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NATIONAL INSTITUTE OF ARTHRITIS, DIABETES, AND DIGESTIVE AND KIDNEY DISEASES
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Z01 AM 52008-03 GBB
Z01 AM 52009-03 GBB
Z01 AM 52010-01 GBB
Z01 AM 53001-12 DDB
Z01 AM 53002-10 DDB
Z01 AM 53004-10 DDB
Z01 AM 53500-09 DDB
Z01 AM 53501-09 DDB
Z01 AM 53503-08 DDB
Z01 AM 53505-07 DDB
Z01 AM 53508-05 DDB
Z01 AM 53509-04 DDB
Z01 AM 53510-03 DDB
Z01 AM 53511-03 DDB
Z01 AM 53512-03 DDB
Z01 AM 53513-02 DDB
Z01 AM 54016-07 DDB
Z01 AM 54017-06 DDB
Z01 AM 54018-06 DDB
Z01 AM 54019-06 DDB
Z01 AM 54020-05 DDB
Z01 AM 54021-05 DDB
Z01 AM 54023-05 DDB
Z01 AM 54024-05 DDB
Z01 AM 54025-04 DDB
Z01 AM 54027-03 DDB
Z01 AM 54028-03 DDB
Z01 AM 54029-02 DDB
Z01 AM 54030-02 DDB
Z01 AM 54031-01 DDB
Z01 AM 54032-01 DDB
Z01 AM 54033-01 DDB
Z01 AM 69000-17 EFS
Z01 AM 69001-13 EFS
Z01 AM 69006-12 EFS
Z01 AM 69009-17 EFS
Z01 AM 69013-01 EFS
Z01 AM 70500-04 CMO
Z01 AM 70501-04 CMO
Z01 AM 70502-04 CMO
Z01 AM 70503-04 CMO
Z01 AM 70505-04 CMO
Z01 AM 70506-02 CMO

INACTIVE PROJECTS

Z01 AM 15105-06 LCDB
Z01 AM 15403-04 LCDB
Z01 AM 19212-06 LC
Z01 AM 21013-05 LEP
Z01 AM 21014-05 LEP
Z01 AM 21017-03 LEP
Z01 AM 25002-09 LCB
Z01 AM 25035-03 LCB
Z01 AM 27007-20 LPB
Z01 AM 29014-22 LCP
Z01 AM 45003-08 CEB
Z01 AM 45024-06 CEB
Z01 AM 45029-03 CEB
Z01 AM 53506-06 DDB
Z01 AM 54026-03 DDB

TERMINATED PROJECTS

Z01 AM 13000-09 MRB
Z01 AM 15001-08 LCDB
Z01 AM 15202-03 LCDB
Z01 AM 15300-17 LCDB
Z01 AM 17005-17 LBM
Z01 AM 19222-05 LC
Z01 AM 19224-04 LC
Z01 AM 19234-02 LC
Z01 AM 25026-05 LCB

TERMINATED PROJECTS (Cont'd)

Z01 AM 25031-04 LCB
Z01 AM 33053-09 LMB
Z01 AM 41029-06 ARB
Z01 AM 45015-12 CEB
Z01 AM 47003-06 DB
Z01 AM 47016-05 DB
Z01 AM 47023-03 DB
Z01 AM 52007-03 GBB
Z01 AM 53504-08 DDB
Z01 AM 70504-04 CMO

DIRECTOR'S SUMMARY

The National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases (NIADDK) achieved a major milestone in 1982 when it was elevated to Bureau status within the NIH. Each of the four extramural disease clusters (Arthritis, Musculoskeletal and Skin Diseases; Diabetes, Endocrinology and Metabolic Diseases; Digestive Diseases and Nutrition; Kidney, Urologic and Hematologic Diseases) achieved new visibility by becoming divisions of the NIADDK.

In more than three decades, since its establishment as an Institute, NIADDK has served the American people through its efforts to elucidate the prevention, diagnosis and better management of a wide range of mostly chronic disorders. Some of these diseases are the leading causes of death in the Nation; others adversely affect the quality of life for those who suffer from them; taken together, they constitute a tremendous direct and indirect drain on the economic resources of the United States.

The wide range of disorders that are the focus of NIADDK's attention include: arthritis and connective tissue diseases, bone and musculoskeletal diseases, skin diseases such as psoriasis and epidermolysis bullosa, diabetes, cystic fibrosis and other metabolic and endocrine disorders; digestive diseases and nutritional disorders; diseases of the kidney and urinary tract, and blood disorders. While this is a truly broad array, there are certain common scientific biomedical denominators in NIADDK's mission.

These programs taken together encompass the various disciplines of internal medicine, less the cardiovascular system, allergy, and infectious diseases. Many of them overlap with a common thread of endocrinology, metabolism and nutrition running throughout. Basic research in endocrinology and metabolism has provided answers to the problems of diabetes, and research directed towards diabetes has led to new insights in other endocrine and metabolic diseases. The fields of metabolism and nutrition overlap, and nutrition and diet therapy are an indispensable part of diabetes, digestive diseases, kidney and blood diseases research, treatment, and prevention. Moreover, in the case of certain metabolic diseases, diet therapy is the only effective treatment available. Autoimmune mechanisms are another major thread connecting different programs of the Bureau. Aberrant autoimmune processes are suspected or implicated in diverse diseases for which NIADDK bears responsibility such as rheumatoid arthritis, insulin-dependent diabetes, Crohn's disease, chronic active hepatitis, primary biliary cirrhosis and the glomerulonephritides.

Hope for more effective treatment and prevention of these diseases lies in basic and clinical investigation into the nature of their interference with the normal function of the body's many complex systems. To carry out this mission, the Institute supports research and training projects in more than 400 non-Federal institutions through the use of grants and contracts. Intramural basic and clinical research is also conducted at facilities on and near the NIH campus at Bethesda, Maryland and at field stations in and near Phoenix, Arizona.

Arthritis and Related Diseases

More than 30 million Americans suffer from the different forms of arthritis, including some that are fatal. The economic cost to the Nation is estimated at over $14 billion annually. The term "arthritis" includes more than 100 different disorders of the joints and connective tissues, including osteoarthritis, rheumatoid arthritis, systemic lupus erythematosus, spinal arthritis, and gout. Arthritis research was the original mandate of NIADDK and continues to receive research and programmatic emphasis. While the underlying causes of most types of arthritis remain elusive, basic research is being pursued into antigens, immune complexes and aberrations in control of immune processes having a possible relation to arthritis, as well as toward expanding our understanding of those viral agents that have been shown to cause some forms of arthritis and related diseases.

A major portion of NIADDK arthritis research involves the discipline of immunology. Aberrant immune reactions are implicated in the inflammatory processes that characterize many rheumatic diseases, and particularly rheumatoid arthritis. Even though the precise etiology of this disease, which afflicts approximately 6 million patients, is as yet unknown, NIADDK-supported research has lead to a substantial clarification of the autoimmune-like pathologic inflammatory changes in affected joint linings which lead to the limitation of motion and deformities in this crippling disease. Consistent with this is the recent finding that total lymphoid irradiation (which decreases the number and activity of immunocompetent lymphocytes) can produce significant improvement, up to 18 months, in active rheumatoid arthritis unresponsive to conventional therapy.

An estimated 19 million Americans suffer from osteoarthritis, which is a progressive degeneration of the joints. Much new information on osteoarthritis has recently emerged from studies in two general areas: biomechanics of cartilage and joints, and environmental influences such as prolonged, repetitive exercise.

A great deal of research effort will continue to focus on systemic lupus erythematosus (SLE), a serious disease of connective tissue that primarily affects young women. The prognosis for patients with this disease has vastly improved in recent years. Evidence is accumulating that SLE involves abnormalities of the body's immune system, and much of NIADDK's current work is directed toward that area. Other lines of research with possible relevance to SLE that are currently being pursued include the influence of sex hormones and the thymus gland. Recent research indicates that male hormones suppress and female hormones enhance the disease process.

The Institute sponsors approximately 21 Multipurpose Arthritis Centers (MACs) located across the entire country, from New Hampshire to Hawaii. These centers are an important focus of basic and clinical research on arthritis, training programs for medical and allied health professionals, and also serve as regional distribution points for the dissemination of arthritis information to both health professionals and patients.

The NIADDK Program for Cooperative Systematic Studies of Rheumatic Diseases is a special initiative designed to accumulate valuable clinical data as a basis for improved health care. This cooperative effort involves 11 clinics, coordinated by a center at the University of Utah.

Musculoskeletal Diseases

More than 40 million people in the United States suffer from bone and joint diseases, fractures, and disorders of the tendons and ligaments, and the loss to the economy in terms of disability and decreased productivity is correspondingly enormous.

Among the musculoskeletal disorders, osteoporosis, a disease characterized by a depletion of bone minerals leading to a loss of bone mass, affects an estimated 10 to 15 million people. Although the underlying factors preceding the development of osteoporosis have not been fully elucidated, investigators have undertaken a 20-year longitudinal study of calcium and bone metabolism in normal volunteers. For those who develop osteoporosis, two forms of therapy will be evaluated: estrogen/androgen administration and calcium supplementation.

A significant portion of NIADDK research is directed toward surgical techniques and materials that now make it possible to replace destroyed hip and other joints with artificial implants. Recent research and development has led to a significant improvement in the long-term prognosis for users of artificial joints.

In another area of musculoskeletal research, work sponsored by NIADDK has been instrumental in the development and refinement of electrical stimulation as a method of healing bone fractures. Investigation into this technique, as well as clinical experience, have shown both short-term safety and efficacy when used within well-defined guidelines for treating nonunion of fractures, failure in fusion surgery, and congenital pseudoarthrosis.

Skin Diseases

Skin diseases are an area of NIADDK concentration where, while only a few conditions are fatal, most of these disorders cause considerable human suffering through discomfort, disfigurement and disability. The medical, psychological, social and economic impacts of cutaneous diseases justify an intense and directed research effort.

Investigations funded by NIADDK are now underway on a wide range of dermatological disorders, including psoriasis, acne, vitiligo, bullous skin diseases, ichthyosis and congenital disorders of keratinization, and eczematous and immunologic skin diseases. Employing newly developed laboratory techniques, or refinements of existing ones, scientists supported by the Institute are attempting to clarify the roles of immunity, allergic response, and metabolic derangements in the causation of a number of these disorders.

Diabetes

Diabetes mellitus is a major health problem that has been diagnosed in over 5.5 million Americans and is undiagnosed in another estimated 5 million individuals who are likely to develop active disease in later life. It is the fifth leading cause of death by disease in the United States, but its full toll is considerably higher, as its vascular complications contribute heavily to the cardiovascular and cerebrovascular death statistics. Passage of the National Diabetes Mellitus Research and Education Act in 1974 led to a marked expansion of research on diabetes, with NIADDK designated as the lead Agency, coordinating work by other NIH components and Federal agencies.

A strong research effort in the area of diabetes has lead to major advances in understanding of the normal mechanisms of insulin synthesis, secretion and action, and their aberration in different forms of diabetes, and disorders of insulin resistance, including obesity.

Other recent research advances include the successful transplantation of pancreatic islets between different species without continuous immunosuppression or short-term rejection of transplanted tissues, the demonstration of the importance of genetic, viral, and immunologic factors in the development of diabetes, the development of synthetic human insulin to the point where it is being subject to clinical trial, and the development and testing of automated insulin delivery systems.

The Institute also supports diabetes research through the Diabetes Research and Training Centers (DRTCs) and the Diabetes-Endocrinology Research Centers (DERCs). The DRTCs, of which there are currently seven, have an important training component for physicians and allied health professionals, in addition to biomedical research. The DERCs are engaged exclusively in research.

Endocrinology

NIADDK's endocrinology program conducts and supports basic and clinical research on the normal and abnormal functioning of the endocrine glands; the structure, function, and mechanism of action of the hormones produced (with particular emphasis on events on the cellular level); the effects of the hormones on various processes in the body; and the factors that relate to or modify the effects of the endocrine system. Recent research has led to an increased understanding of the effect of hormones on the normal processes and dysfunctions related to metabolism, growth, reproduction, aging, cardiovascular-renal function and nutrition.

Among these research advances is identification and synthesis of the hormone corticotropin releasing factor (CRF) which originates in the hypothalamus and plays a vital role in the body's response to stress. Other studies, involving recombinant DNA techniques, have made possible the development of synthetic human growth hormone, which is currently undergoing a clinical trial to test its effect, potency, and any possible side effects. Until the mass production of human growth hormone from this source is begun, human pituitary glands extracted at autopsy will remain the only source of providing the needed supply. For many years, the NIADDK has sponsored the National Hormone Distribution Program which, through the cooperation of the College of American Pathologists collects the needed pituitary glands from hospitals and medical centers throughout the country.

Metabolic Diseases

Research advances in the field of metabolic diseases have included the development of improved, earlier diagnosis and clinical management of patient cystic fibrosis, thus dramatically increasing their life expectancy. Major efforts are now devoted to development of reliable tests which would identify carriers of cystic fibrosis and which would permit diagnosis of the disease in utero.

From 30 to 40 percent of all admissions to children's hospitals are attributable to inborn errors of metabolism, and thus the research pursued in this area by NIADDK is of great importance. Many patients in state institutions for the mentally retarded have inborn errors of metabolism (with consequent brain damage) thus contributing to the high cost of care for mental patients. The research areas relevant to the program's goals include normal and pathological metabolic pathways, enzyme structure and function, cellular oxidations and biological membranes, synthesis and biosynthesis of relevant biological substances, and the utilization of modern instrumentation in metabolic disease research.

Digestive Diseases

The NIADDK has the primary responsibility for Federal research programs aimed at implementing the long-range plan recommended by the National Commission on Digestive Diseases. Each year, more Americans are hospitalized for digestive disorders than for any other group of diseases, resulting in medical costs to the nation of approximately $35 billion annually. In the nation, 20 million people are affected by chronic digestive conditions. As new knowledge is gained from basic and clinical research in the anatomy, histology, physiology, endocrinology, and biochemistry of the digestive tract, there is hope that morbidity and mortality can be lowered, and the economic burden reduced.

NIADDK sponsors research in diseases of the esophagus, stomach, pancreas, salivary glands, liver, biliary tract, and small and large intestines. Major digestive diseases studied include esophagitis, peptic ulcer, diverticulitis, infectious diarrhea, ulcerative colitis, malabsorption, hepatitis, cirrhosis, and others. An exciting recent discovery has been the finding that integration of hepatitis-B virus DNA into the genetic structure of liver cells may precede the development of manifest cancer by several years. This suggests that liver biopsy specimens may be useful in identifying hepatitis-B virus carriers who are at high risk of developing primary liver cancer.

Another key area is the search for alternatives to surgery in the treatment of gallstones. Results of the National Cooperative Gallstone Study, a multicenter clinical trial, provided valuable information on the effectiveness, safety, and limitations of chenodeoxycholic acid (chenodiol) in the dissolution of cholesterol gallstones. Results indicated that chenodiol can be an effective treatment in certain selected patients.

Upper gastrointestinal hemorrhage, which is a serious medical problem in the United States, has been the focal point of considerable attention from NIADDK. In addition to the consensus development conference on the diagnostic and therapeutic application of fiberoptic endoscopy which was held at the NIH in August 1980, a workshop was held more recently on the use of recently developed, highly sophisticated endoscopic methods in basic studies, and to demonstrate their effectiveness as a tool for reaching areas previously inaccessible to conventional endoscopes.

Nutrition

Nutrition is an important area of biomedical research because it plays a key role in the maintenance of health and the prevention of disease. The nutrition program at NIADDK emphasizes the following areas: prevention and control of obesity; early detection of malnutrition and improved nutritional support of hospital patients; the dietary role of fiber; health consequences of trace mineral deficiencies; the role of nutrition in immune competence and infection; interaction of nutrients and drugs; and environmental and host factors that affect nutritional requirements.

Noteworthy recent accomplishments supported by the NIADDK nutrition program include the discovery that a decrease in the number of insulin receptors on fat cells is the principal cause of insulin resistance in human obesity, and the prospect that certain keto acid analogues of branched-chain amino acids may offer new approaches to nutritional therapy for patients with end-stage renal disease. The supportive technique of total parenteral nutrition has been further perfected to the point where many patients who have lost most or all of their intestinal function because of surgery, trauma, or disease, can continue a near-normal existence without manifest nutritional or functional impairment.

The Institute also collaborates with other NIH components through the NIH Nutrition Coordinating Committee to stimulate cooperative efforts in this area, which often crosses strict oganizational lines.

Kidney and Urologic Diseases

The primary emphasis of the kidney and urologic diseases program is support of research into the underlying mechanisms of these diseases in order to facilitate their efficacious treatment. Because of the complex pathophysiologic nature of the program's disease interests, a major effort has been to encourage scientists in many different disciplines and specialty areas to focus upon such special concerns as urolithiasis, glomerulonephritis, and benign prostatic hyperplasia. Other areas of study include nephrosis, interstitial nephritis, polycystic kidney disease, kidney and bladder stones, diabetic nephropathy, immune disorders of the kidney, and analgesic-associated and other toxic nephropathies. End-stage renal disease and uremia constitute another important research area.

Recent advances sponsored by NIADDK in this area include the development of continuous ambulatory peritoneal dialysis (CAPD), a method of treating end-stage renal disease that usually does not require dietary restrictions; the demonstration of the effectiveness of plasmapheresis, in coordination with immunosuppressive therapy, in the treatment of Goodpasture's syndrome; the development of new surgical techniques to permit restoration of damaged or congenitally absent ureters, which successfully establish valve action to prevent urine reflux; and the successful development of selective drug and diet therapy of specific metabolic derangements (uricosuria, hypercalciuria and others) which can prevent the recurrence of kidney and urinary tract stones in a great majority of patients with urolithiasis.

Three clinical trials in the kidney/urology area are currently underway, all supported by the research grant mechanism: 1) medical vs. surgical treatment of vesicoureteral reflux; 2) improved treatment of glomerulonephritis; and 3) plasmapheresis in the treatment of lupus nephritis.

Blood Diseases

The hematology program at NIADDK supports investigations into the basic aspects of normal and abnormal blood cell components and function, and the pathogenesis, diagnosis, and treatment of diseases of the blood. Areas studied include hemoglobin structure and genetics; blood cell production; iron metabolism, transport and storage; iron overload and deficiency; and white blood cell metabolism and function.

Advances achieved through support by the NIADDK hematology program have not only improved the diagnosis and treatment of blood diseases, but have also benefited research falling under other NIH programs or other NIH Institutes. An example of this is the finding that the first-year functional survival of transplanted kidneys is vastly improved if the graft recipient has received multiple blood transfusions during the period preceding the transplant operation. Other NIADDK-supported studies have led to the development of greatly improved, safe and sensitive methods for the prenatal diagnosis of Cooley's anemia and sickle cell disease.

Equal Employment Opportunity Program

The Bureau's EEO program has operated under a transitional directorship for most of fiscal year 1982. Efforts were therefore focused mainly on outreach activities and employee assistance in the forms of education and training, placement, and informal resolution of complaints.

Internal Initiatives:

Annual EEO Meeting - In the fall of 1981, a Sexual Harassment seminar was provided for Bureau employees at the Annual EEO Meeting. A special achievement award was presented to the Director for his support and encouragement toward reaching Equal Employment goals and objectives. Members of the EEO Advisory Committee also received certificates for special contributions in this area.

The Bureau supports the development of disadvantaged minority students through the Stay-in-School program which allows them to work part time while pursuing their education. The Bureau supports such positions, in administrative and extramural positions, and in the intramural program.

A monthly newsletter is prepared and distributed by the EEO Office apprising all Bureau employees of matters of importance that impact on them. Personnel policies and procedures are explained in simple terms with the advice and consultation of the Personnel Branch.

The EEO Program has recently appointed a Federal Women's Program Coordinator for the Bureau. This component will allow for continued leadership and representation for women's special concerns and offer an opportunity for special initiatives directed toward improving the status of women in the Bureau.

Tissue culture classes continue to be offered in the Division of Intramural Research for all interested employees who wish to pursue a career or expand their skills in the field of biomedical science.

Outreach Activities:

Through the Minority Biomedical Support Program and in conjunction with the Division of Research Resources, funds are committed to support projects designed to strengthen the biomedical science capabilities of minority institutions. These funds are used to support undergraduate students, as well as graduate, post-doctoral staff and faculty positions.

The Minority Access to Research Careers (MARC) Program interagency agreement with the National Institute of General Medical Sciences allows the Institute to increase the number of minority scientists engaged in biomedical research. Funds are available for pre-doctoral faculty fellowships, visiting scientists, and honors undergraduate training.

A New NIH Initiative which allows NIH scientists to visit minority institutions and participate by giving scientific lectures or advising students on careers in biomedical sciences, has begun this year. Thus far, a number of NIADDK scientists have indicated their interest in participating in this program which not only provides a means of attracting and recruiting talented minorities into biomedical research, but also provides an avenue for participating colleges and universities to become directly aware of the opportunities in research at the Bureau and the NIH in general, encouraging faculty and students to interact with and learn from NIH intramural research scientists.

The EEO Office continues its distribution of scientific journals to various minority colleges and universities, donated by NIADDK staff. These contributions to staff faculty, department libraries, and individual students, enable these colleges to better prepare minority youth for successful careers in the sciences.

The Bureau's EEO staff participates in the Black Colleges Initiative which originated by Executive Order #12232 under the Carter Administration in 1980 to overcome the effects of discriminatory treatment and to strengthen and expand the capacity of historically black colleges and universities to provide quality education and to participate in Federally sponsored programs. Executive Order #12320, dated September 15, 1981, was signed by President Reagan and superseded the previous Order. Although differing in the method of implementation and the inclusion of the private sector, the new Order has essentially the same objectives.

OFFICE OF PROGRAM ACTIVITIES AND EVALUATION

The Office of Program Activities and Evaluation (OPAE) has the responsibility for the Bureau's prevention-related, evaluation, technology assessment, technology transfer, and international activities. OPAE also provides staff support to the immediate Office of the Director, NIADDK. OPAE serves as Bureau liaison with several NIH offices and committees, including the Office of Disease Prevention, the Office of Medical Applications of Research (OMAR), the NIH Office of Program Planning and Evaluation (for evaluation-related activities) and the NIH Committee on Disease Prevention-Health Promotion. The Office of Program Activities and Evaluation is also responsible for the Annual Report of the Director, NIADDK, which covers overall Bureau activities, specific program efforts and research accomplishments and directions.

Disease Prevention

The Office of Program Activities and Evaluation is the focal point for Bureau activities related to prevention. These include coordination, analysis, periodic and special reporting, information dissemination, conference sponsorship, external representation, and execution of evaluation-related and prevention-related studies and projects.

OPAE surveys ongoing Bureau activities related to disease prevention, as well as provides a retrospective analysis of active NIADDK grants and contracts to identify those with a prevention component. This review of current grants and contracts was completed in 1982. In addition, OPAE is developing a coding system for future awards to identify those with components relating to prevention. This activity is being carried out in close collaboration with the Office of Program Planning and Analysis and the Division of Extramural Activities.

In close collaboration with the various Divisions, OPAE identifies research topics with prevention potential such as:

- "Sports Medicine" (Prevention of injuries)
- Osteoporosis
- Prevention of Type II Diabetes
- Prevention of Dwarfism and Normal-Variant Short Stature
- Prevention of Recurring Peptic Ulcer
- Diverticulosis and Diverticulitis
- Prevention of Recurring Cholelithiasis
- Obesity
- Analgesic-Associated Renal Failure

- Nutrition

- Prevention of Recurring Urolithiasis

- Benign Prostatic Hyperplasia

OPAE also has responsibility for planning and organizing prevention-related conferences and workshops.

OPAE produces prevention-related materials for inclusion in pertinent Bureau and NIH documents. Examples of this are the preparation of a section on prevention and related activities for the annual NIADDK budget narrative and the 3-year research report, a section on prevention in the opening statement of the Bureau Director at appropriations committee hearings, a special chapter on prevention-related research in the Annual Report of the Director of NIADDK, and preparation of NIADDK's portion of the Annual NIH Report on Disease Prevention. In addition, in FY 1983 OPAE will prepare a special report detailing the Bureau's activities related to prevention.

Program Activities

OPAE is responsible for preparation of the Annual Report of the Director, NIADDK. The First Annual Report was delivered by the contractor in 1982, and is in the process of being printed by the Government Printing Office. The first of its kind, this Congressionally mandated report will be disseminated very widely to the Bureau's diverse professional and lay constituencies as well as to the Congress. Preparation of the Second Report is well underway. The Report provides an overview of the Bureau research and training efforts in the previous year, emphasizing initiatives and accomplishments in all the Divisions. The First Annual Report also provided a 30-year retrospective on the founding and development of NIADDK as a major component of the NIH.

The Office of Program Activities and Evaluation provides direct staff support to the Director, NIADDK, for a wide range of activities. These include providing the record of the bi-weekly meeting of the Associate Directors of the Bureau and performing liaison duties with the Office of Medical Applications of Research (OMAR) for consensus conferences sponsored by NIADDK. OPAE also provides liaison for the Bureau with respect to international activities pursued by the NIH.

Evaluation

OPAE prepares the Bureau's Annual Evaluation Plan which describes the context in which evaluation activities are carried on at NIADDK, and sets forth its evaluation strategy, a description of evaluation projects newly proposed, a summary of ongoing projects and a notice of projects recently completed. OPAE acts as liaison for NIADDK with the NIH Office of Program Planning and Evaluation respecting matters pertaining to program evaluation.

OPAE took steps in 1982 to implement modifications to the Evaluation Plan required by a new emphasis on policy questions and objectives in each program of the Bureau. While performance or policy issues for all 10 categorical programs at NIADDK have been (or are in the process of being) identified, two areas in particular are the subject of studies that are expected to produce final or interim reports in 1982. These are an evaluation of the research needs in the field of hematology and how they can best be addressed by the program at NIADDK, and a project to identify and develop methods of evaluating NIADDK center grant programs.

In addition to the overall plan for evaluating the categorical programs, the Bureau has submitted proposals for the following projects to begin in FY 83.

1) a cross-cutting study of diabetes, endocrinology and metabolic diseases research grant applications to compare peer review outcomes of proposed research defined as either clinical or basic;

2) the development of a strategy for assessing the activities of the National Digestive Diseases Education and Information Clearinghouse; and

3) an evaluation of the effect on diabetes treatment and management by primary care physicians of a new manual ("Guide to the Treatment and Prevention of Five Complications of Diabetes") produced under the auspices of the National Diabetes Advisory Board and disseminated by NIADDK's Diabetes Information Clearinghouse.

Technology Assessment and Transfer

OPAE is the locus within NIADDK for technology assessment and technology transfer activities. These include providing state-of-the-art information to OMAR for communication to the Health Care Financing Administration regarding Medicare coverage for certain medical, surgical, and diagnostic procedures and treatments, along with computerized bibliographies of relevant scientific literature. Some requests for information the office has responded to have included the following topics:

- Electromagnetic Treatment of Bone Fractures
- Liver Transplantation as a Treatment for Liver Disease
- Apheresis for the Treatment of Goodpasture's Syndrome and Membranous and Proliferative Glomerulonephritides
- External Insulin Infusion Pump for Treatment of Diabetes Mellitus
- Pancreas Transplantation for the Treatment of Diabetes Mellitus

OPAE is also the Bureau liaison with OMAR regarding patent rights to inventions arising out of research supported by NIADDK. Some inventions for which in 1982 the office obtained expert advice regarding patentability were the use of Cholesterol Sulfotransferase to select "Keratolytic" agents, the wavelength controlled production of Previtamin D3, and a procedure for the facile enzymatic synthesis of fatty ACYL CoA's.

The consensus conferences sponsored and coordinated by OPAE, pertaining both to prevention and other areas within the purview of NIADDK, are another example of office responsibilities with respect to technology assessment and transfer.

OFFICE OF HEALTH RESEARCH REPORTS

The Office of Health Research Reports (OHRR), formerly the Office of Scientific and Technical Reports, prepares, produces and disseminates information about the varied research and programs of the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases (NIADDK). This information takes many forms: brochures, pamphlets, fact sheets, background papers, announcements news releases, reports to the Congress, responses to public and congressional inquiries, exhibits at scientific meetings, and importantly, contact with print and broadcast media representatives.

On April 23, 1982, the Secretary of the Department of Health and Human Services (HHS) created the Division of Arthritis, Musculoskeletal, and Skin Diseases; the Division of Diabetes, Endocrinology, and Metabolic Diseases; the Division of Digestive Diseases and Nutrition; the Division of Kidney, Urologic, and Hematologic Diseases; the Division of Intramural Research; and the Division of Extramural Programs. These new divisions will be given more focused responsibility, and greater prominence and authority for the national research effort in their areas.

CONGRESSIONAL INQUIRIES

The Office of Health Research Reports coordinates and produces responses to all "controlled" correspondence referred to the Institute from the Congress and the White House. Because of active public interest in a wide-ranging number of subjects within its research purview, NIADDK receives a large volume of these requests.

During the period May 1981 through May 1982, OHRR prepared 158 written responses to inquiries from Senators, Congressmen, the Secretary of DHHS, and the White House.

Telephone inquiries from congressional staff are coordinated by distribution of "records of call" to appropriate members of Institute staff.

PUBLIC INQUIRIES

A significant portion of the U.S. public is directly concerned with the disease areas under investigation by NIADDK. An estimated 60 million people in the United States are victims of arthritis, diabetes, and digestive diseases alone. Kidney diseases still claims the lives of 45,000 Americans annually. During the period May 1981 through May 1982, OHRR received approximately 8,333 telephone inquiries from patients, their relatives and friends, students, allied health professionals, physicians, and scientists. In addition, 405 letters were written in response to public inquiries, exclusive of requests for publications or congressional inquiries, an increase of 30 percent over 1980-81 figures.

Requests for information dealt with questions about controversial forms of therapy, how to become a patient in NIADDK research studies, financial aid, physician referral, accepted diagnosis and treatment procedures, proposed "cures" for certain diseases, and NIADDK research grant and contract programs.

PRESS ACTIVITIES

Contact with representatives of print media is an integral part of OHRR's public information program. In support of the established policy to provide information about NIADDK's programs and activities, OHRR reaches a variety of media outlets through personal contact and distribution of research highlights and announcements.

OHRR prepared a number of announcements during the past year, including Dr. Lester B. Salans appointed as Acting Director, NIADDK; HHS Secretary Richard S. Schweiker announced the appointment of members to the National Arthritis Advisory Board, the National Diabetes Advisory Board, and the National Digestive Diseases Advisory Board; Availability of National Diabetes Advisory Board 4th Annual Report "Diabetes in the 1980's: Challenges for the Future"; Digestive Diseases Clearinghouse Established; Digestive Diseases Advisory Board Appointed; Five New Members Appointed to NIADDK Advisory Board; New Findings in Sickle Cell Anemia Research; Puva Therapy for Psoriasis; Synthesis of Medical Opiates; Glucagon Blockers Lower Blood Glucose in Diabetic Rats; Major Prevention Initiative in Diabetes to Begin; Pharmacology of Benzodiazepines; NIH Consensus Development Conference: Hip Joint Replacement; Aerobic Exercise Training of Artificial Kidney Patients; and HHS Secretary Announces NIADDK Converted to a Bureau. Announcement was made by HHS of the appointment of Dr. Salans as Director of NIADDK on June 17, 1982.

A list of newspaper and magazine articles that covered NIADDK programs is available in OHRR.

RADIO AND TELEVISION

A number of scientists were featured this year on radio and television broadcasts, both locally and nationally.

Dr. Lawrence E. Shulman was the guest on the local NBC affiliate's Tony Brown show in October 1981.

On June 29, 1982, David Culhane of CBS network evening news interviewed Dr. John Decker, chief, Arthritis and Rheumatism Branch, on drug treatment and research on rheumatism and arthritis. Dr. Michael Miller, clinical associate in the Arthritis and Rheumatism Branch, Division of Intramural Research, was interviewed about juvenile arthritis on radio station WGMS in February 1982.

Dr. Lester B. Salans was interviewed by CBS network news correspondent Lem Tucker about fat metabolism and diabetes on April 15, 1982. Dr. Phillip Gordon, clinical director, Division of Intramural Research, was interviewed about hypoglycemia on WGMS in May 1982.

Dr. Harold Roth, associate director, Division of Digestive Diseases and Nutrition, was interviewed about overindulgence in food and drink on Cox TV broadcasting in December 1981. He was also interviewed about gallbladder disease on WGMS in October 1982 and was featured by Steve Gendel of the local CBS affiliate Channel 9, in May 1982, on the subjects of gallstones and ulcers. Dr. Jerry Gardner, chief, Digestive Diseases Branch, Division of Intramural Research, was interviewed about ulcers on WGMS in February 1982.

Approximately 200 other radio stations throughout the country subscribe to the NIH interviews that are run on WGMS locally. In April 1982, more than 2,000 radio stations received 30 second and 60 second public service announcements on peptic ulcers.

THE NIH RECORD

A primary source of communication is through The NIH Record, which is distributed to NIH employees, wire services, local news media, science writers and editors, and scientists in the United States and abroad. As a result of this continuing emphasis, the number of items about NIADDK's activities increased to 62 in 1981-82. A list of NIADDK articles in The NIH Record is available in OHRR.

During the past year, articles also covered retirements and resignations of five individuals.

SPECIAL PROJECTS

In December 1982, OHRR represented NIADDK at the White House Conference on Aging, attended by 4,000 people, by designing and staffing a new exhibit on arthritis. A volunteer from the local Arthritis Foundation chapter was recruited to assist OHRR staff at the conference, and appropriate Institute and Arthritis Foundation materials were distributed.

In March 1982, NIADDK cosponsored an NIH Consensus Development Conference with the Office for Medical Applications of Research. OHRR staff edited and produced an abstract of the presentations; developed materials to advertise the program; pursued media contacts before, during, and after the conference; and assisted in the preparation of the consensus statement.

In the past year, NIADDK has put additional emphasis on cooperative activities with voluntary health organizations, and advised and assisted clearinghouse representatives in actively soliciting private sector entities to participate in joint projects.

OHRR attempted to coordinate clearinghouse operations at NIH and NIADDK by establishing an NIH Clearinghouse Resource Group with the National Cancer Institute. OHRR also represented NIH on the PHS Task Force on Clearinghouses, and with the National Diabetes Information Clearinghouse made presentations on process evaluation to the PHS-sponsored seminar on evaluation in public affairs held at NIH on June 30, 1982.

EXHIBITS

OHRR has responsibility for coordinating the exhibit program for NIADDK, designed primarily for a scientific audience. During the past year, five major exhibits were viewed at eight medical and scientific meetings throughout the United States by more than 55,000 physicians, scientists, and allied health professionals.

During the period May 1981 to May 1982, Institute exhibits were displayed at meetings of the American Diabetes Association, American Gastroenterological Association during Digestive Diseases Week, the American Society for Artificial Internal Organs, the American Urological Association, the American Public Health Association and the Lupus Foundation. Appropriate publications were distributed by OHRR staff members who accompanied exhibits.

Lack of funds to develop and transport exhibits has curtailed their use and led to a re-evaluation of their purpose. In the meantime, the focus of OHRR information activities has shifted to "covering" the reports of grantees and intramural scientists made at 1982 meetings of the AGA, PANLAR, FASEB, and ADA, rather than staffing exhibits.

PUBLICATIONS PROGRAM

During Fiscal Year 1982, OHRR continued its publications program on a limited basis due to the OMB-imposed moratorium on the production and printing of new publications and periodicals by the Federal Government.

Exemptions are rare, and thus OHRR has pursued in-house photocopying of short fact sheets, such as "Therapies for People with Arthritis," "Treatment of Psoriasis," "A Statistical Look at Diabetes," and a report of the national study of plasmapheresis in lupus nephritis. In addition, OHRR has focused on obtaining information materials from a variety of non-government sources. For example, a limited number of Arthritis Foundation publications are available for distribution and voluntary organizations in digestive diseases are providing OHRR with materials. Permission to reprint articles in the lay and trade press have been received in several instances. Nevertheless, approximately 14,000 existing publications were distributed to the public from May 1981 through May 1982.

A new brochure entitled: "How to Cope with Arthritis," which received approval prior to the OMB moratorium, was produced and distributed to the lay public. A pamphlet entitled, "Understanding Paget's Disease," has been completed and is awaiting funding for production. A pamphlet on osteoporosis has been reviewed scientifically and while funding is unavailable, is being pretested with a sample audience.

Publication and editorial assistance was provided to program personnel on the conference proceedings and bibliography for the Fourth International Conference on Epidemiology of the Rheumatic Diseases; on the Fourth Annual Report of the National Diabetes Advisory Board; on the Third Annual Report of the Digestive Diseases Coordinating Committee, Fiscal Years 1979 and 1980; and on the Arthritis Information Clearinghouse Patient Education Resources Catalog.

ANNUAL REPORT FOR THE

DIVISION OF EXTRAMURAL ACTIVITIES

OFFICE OF THE ASSOCIATE DIRECTOR - Dr. George T. Brooks

The Division of Extramural Activities (DEA) and the Office of the Associate Director (OAD-DEA) has responded to a wide variety of activities in FY 82. This reflects both the coordinating and supportive roles of the OAD-DEA as well as the increasing number of responsibilities being placed upon the office and its four branches: Grants Management, Contracts, Scientific Programs Information and Review. The separate branch reports, which follow, detail accomplishments, challenges, changes and new requirements as they pertain to the individual branches. Those activities emanating from the OAD-DEA and those having impact either across the branches of this Division or across all extramural Divisions are highlighted in this section.

The DEA has recognized for some time, the need for dissemination of grant, contract, and budget information. In order to formulate, collect, analyze and disseminate this variety of information the OAD-DEA has expanded to include an Assistant Director and a Program Analyst.

The Assistant Director has been given primary responsibility for: the coordination and execution of the NIADDK Extramural budget, development of procedures and policies which assure effective coordination and utilization of all shared extramural activities; shares with the Director, DEA, the management, direction and supervision of the four branches playing a major role in the supervision of the Grants Management Branch and the Contracts Branch; and developes position statements on proposed NIH policies, procedures, and manual issuances.

In order to provide the Institute Director, other Division Directors and the Advisory Council with information that may be used for policy development, procedural changes, special initiatives, modifications of grant mechanisms etc, the OAD has begun the process of disseminating analyses of a variety of grant and contract characteristics. To date the following studies have been made:

An indepth study of the status and ability of M.D. researchers to compete for and retain research support.

A study of the current status and past history of the institute's large multidisciplinary projects, particularly program projects.

A trend study of the institute's regular research support mechanisms depicting overall funding levels (Total Costs and Direct Costs) showing individual budget category distributions (e.g. personnel, supplies, etc.)

A study of NIADDK applications and awards for the last five years showing the proportion of competing awards.

A study of NIADDK regular research awards showing the average total cost award for the last five years per mechanism in terms of both current dollars and constant dollars.

To allow for more flexibility and responsiveness in the operation of the Scientific Programs Information Branch, the OAD-DEA has instituted procedural changes in the scientific coding of both non-competing and competing applications. These changes have significantly reduced the time required for coding.

A key member of the DEA, the Chief of the Review Branch, has departed the Institute, accepting another position in the Division of Research Grants. Considering the importance of this position to the DEA and the Institute, a major priority of this office is the recruitment of a well qualified replacement. In the interim, the Associate Director, DEA has assumed the role of acting Chief of the Review Branch.

While the two problems identified in last years report (personnel and space) continue to limit the effectiveness of our operation, the lack of adequate space is the most severe.

GRANTS MANAGEMENT BRANCH

This year, in May, the NIADDK funded a new clinical trial in insulin-dependent diabetes mellitus. The trial consists of four separate phases (planning, limited feasibility testing, full-scale clinical trial, and data analysis reporting). A unique feature of this clinical trial is the use of the Cooperative Agreement Grant as the funding mechanism. Use of this instrument represents the first Departmental authorization for the NIH to utilize this new funding mechanism for the support of a research project of this nature. It is also a first for the Grants Management Branch.

The number of grants funded by the Co-Funding method has increased considerably over the past year. We have continued to use the Co-Funding method to award certain Conference grants (R13) as well as to contribute to the Minority Biomedical Support Program (S06). One of the most striking features that has emerged over the past year has been an increase in the number of Institutes participating in the support of a single grant. Last year most Co-Funding was accomplished by agreements between two or three Institutes. This year we have funded grants that have had from five to seven participating Institutes, thereby adding to our administrative responsiblities.

Operating under Continuing Resolutions thus far this year has increased significantly our management requirements because of the need for various revisions to our awarded grants. During the second Continuing Resolution we applied a 12% reduction from the President's March 1981 request for FY 1982 appropriations to our grants programs. During the third Continuing Resolution we had to restore 8.5% of the previous 12% reduction. Further our center programs were reduced on an average of 7%. The following actions were taken to comply with the directives of the third Continuing Resolution with regards to training: The instituional allowance associated with individual fellowships was reduced to $3,000; the corresponding allowance for institutional fellowship awards (training grants) was reduced to $2,500 per postdoctoral trainee and $1,500 per predoctoral trainee. All of the above mentioned limitations apply to awards made with FY 1982 funds. No reductions have been made to awards issued prior to October 1, 1981, or in amounts recommended for approved grants in future years.

Effective January 4, 1982, for-profit organizations became eligible to apply for assistance awards (research grants and cooperative agreements) under most sections of the Public Health Service Act. For the most part, the existing body of policies, procedures and guidelines that pertain to non-profit grantees will also apply to for-profit grantees; however, there are some differences with respect to indirect cost rate negotiation, cost-sharing, property management, and program income, all of which must be included, where appropriate, in the terms and conditions of an award. Thus far the NIH has received only about five applications from for-profit institutions, none of which was assigned to the NIADDK.

In FY 82 we hope to install and activate what is known as the interactive awards system which allows the Bureaus, Institutes, or divisions, (BID's) to prepare their own Notice of Grant Awards on site. Such action can be accomplished with CRT terminals (NIH Delta 7000) and printers (CT-45) which will be located within our branch space. The completed grants management/ICMS worksheet, or a previously issued award (for a revised award) will be the source document for these award notices. The system has two parts. Part one selects from the IMPAC file the records with the data to be used in the preparation of the award; while part two displays the data allowing the operator to review, change, and add new data (budget categories etc.). One or more awards may be produced by our Branch in a given day. The data entered through the Interactive Awards System will be used to update the IMPAC system each night. These updates will produce the Approval lists which will be distributed to our Branch from the data control point. We expect the sytem to be time saving and cost efficient.

CONTRACT MANAGEMENT BRANCH

Small Business Administration (SBA) activity emerged as the single most significant development. Much time and effort was expended convincing SBA officials that clinical trials such as PUVA and the Diabetes Coordinating Center were not amendable to small business set-asides. Future procurements will also be challenged; one solution is to formulate explicit work scopes that precisely define involvement of requisite medical expertise. However, we still anticipate that NIH's presolicitation review mechanism will result in more sources sought announcements and requests for proposals under the SBA's guidelines for restricted competition.

Justifications for noncompetitive procurements (JNCPs) are being screened with the intent of conversion to competition whenever possible. Lead time for JNCPs normally ranges from several weeks to several months, contingent upon the echelon of concurrence and approval. Those with estimated costs of up to five hundred thousand can be approved at the NIH level; signature by the Assistant Secretary is required for larger amounts. As of this year, so-called administrative extensions of less than six months duration cannot be effected without a JNCP if additional funds are involved.

Administrative procedures have been revised to include submission by project officers of: (1) technical questionnaires when offeror's proposals are subjected to cost/price analysis by the Financial Advisory Services Branch; (2) review and approval of contract expenditures; and (3) interim advisory reports on scientific progress prior to modifying existing awards to provide for continued performance.

Due to recent audit criticism, the NIH Board of Contract Awards (BCA) commenced issuing more substantive objections. Such objections prior to award may require renegotiation. All must be resolved before a contract can be executed. Consequently, funds cannot be obligated until mutual agreement is reached between all parties. Although BCA reviews have always been extensive, now more emphasis is being placed on definitive descriptions of work and reporting requirements. The objective is to eliminate ambiguity since defective specifications can result in claims against the Government.

Overall economic conditions generated keen competition among offerors in the commercial marketplace. One tangible outcome is an increase in the number of formal protests/debriefings, usually with FOIA implications.

From a volume standpoint, contract activity did not expand appreciably. However, current projections do not indicate further erosion. The loss of another negotiator places more work on remaining staff. Efforts were made to streamline operations in accordance with functional phases, thereby reducing redundancy and diffused responsibility. The goal is continued timely support consistent with available resources.

SCIENTIFIC PROGRAMS INFORMATION BRANCH (SPIB)

The Branch provides information and information services regarding NIADDK programs, particularly extramural programs, to administrative and scientific officials. These services include but are not limited to: analyses and evaluation of ongoing biomedical research activities; maintenance of a computer - based scientific and management information system (MIS); special and recurring reports on grant, contract and intramural programs; maintenance of the NIADDK scientific thesaurus; scientific classification of new grant applications to appropriate program areas; maintenance of accurate information on NIADDK grants in the IMPAC system; recording the NADDK Advisory Council actions; and preparation of approval reports for each Council meeting.

Prior to this year SPIB was the Program Evaluation Branch.

To facilitate the use of SPIB's information services, we wrote a Users Guide. The initial distribution of almost 100 of these books went to NIADDK staff, at all levels, and to other interested parties. The Users Guide is divided into 5 major sections. In summary, these are:

- Introduction - to the branch, to the assignment guidelines and to the grant and contract numbering systems and definitions.
- Program Area Assignment Guidelines - the detailed guidelines, based on DRG's Referral Handbook, for all Programs in each Division.
- Information Systems - definitions of systems and the information in them that we use to answer requests.
- Scientific Codebook - besides the entire 4 axes codebook, it contains the current and historical program area designations, shows how the coding is done and includes the DMCC subject categories.
- Reports - explains how to request a report and includes a request form, details the special and recurring reports with sample pages and gives examples of subjects that have been previously reported.

The Users Guide is designed to be easily revised. The text and distribution list are stored electronically.

Major changes in the scientific coding procedures have been implemented. Grant applications, both competing and non-competing are no longer coded at time of receipt. These changes are summarized below:

- Non-competing - those with 3/1/82 and later begin dates are no longer coded. We developed a computer program that automatically moves the coding from the prior year to the current record. Exceptions to this procedure are brought to our attention by program staff, when the progress report has changes in scientific content. To this end, we designed a checklist which they complete and we use to modify the record.

- Competing - beginning with the 10/82 council round these are coded when the IRG recommendations are available. Applications that are disapproved or have poor priorities are then scientifically coded. As grants are funded we will use the CRISP indexing.

Already, these changes have significantly decreased and leveled the scientific coding workload. It is too soon to forecast the results of using CRISP indexing, but we think they will be positive; in saving time, eliminating duplication of effort and providing a good cross-walk between the CRISP and AM scientific systems.

Discipline, Speciality, Field (DSF) codes are assigned to each fellowship, career and training grant application and to each trainee.

With the documentation and assistance of the budget office and the initial programming efforts of DCRT staff, we have automated 2 versions of a fiscal planning model (FPM). Previously, the model was constructed manually by a budget analyst for each major program and division. Using 'Wylbur' the analyst can now call in a FPM and manipulate it at his computer terminal. We maintain the models and modify them as needed.

This year most computer programs that retrieve grant information have been modified to accommodate the establishment of Institute divisions in place of clusters.

Also, this year, in place of massive monthly distribution of recurring reports to many NIADDK offices, we are providing these reports on demand. This has meant rewriting the computer programs to generate only one program or one division. The initial reports available by program or division under the new system are:

- Paid grants, year to date
- Active grants
- Non-competing commitments
- Competing unpaid applications

Soon to be added are pre and post review cycle reports. We expect that this system will even out and perhaps decrease our report distribution workload. Furthermore, each office has an opportunity to request modifications to these reports to suit their individual needs.

SPIB continues to provide information services but we find ourselves in a catch - 22 situation. The Institute's priorities from us are for increased fiscal and scientific information and analyses, and the establishment of user friendly access to this information. On the other hand, we have lost 2 key professional technical information specialists with no replacements in sight and have been unable to recruit for a high level programmer to create and maintain user friendly computer programs.

With current resources, including those outside the branch our services must continue near present levels with minor expansion in identified high priority areas.

REVIEW BRANCH

The Review Branch is responsible for the merit review of applications assigned to the NIADDK for comprehensive center grants, core center grants, program projects, training grants, clinical investigator awards, contracts, cooperative agreements, and cooperative clinical studies. The Executive Secretary in carrying out the Institute competitive review of these applications encourages both program and grants management staff input into the process. Such input insures a more thorough preparation for site visits and aids the Executive Secretaries in the selection of site visitors.

Though the number of applications reviewed by the Review Branch has remained large and has continued to tax our limited resources, our review staff has continued to conduct quality merit reviews. During this fiscal year, our consultants reviewed grant proposals (see Table 1) requesting a total of 32.9 million dollars. This resulted in recommendations for approval of over sixty percent of the funds requested or 20.3 million dollars.

TABLE 1. REVIEW BRANCH - FISCAL YEAR 1982

Activity Code	COUNCIL October '81	 January '82	 May '82	TOTAL
P01	15	1	9	25
K08	10	1	13	24
T32	17	13	25	55
P30, P60	6	6	4	16
N01	8	4	0	12
Other	67	2	1	70
TOTAL	123	27	52	202

P01 = Program Projects
T32 = Institutional Fellowships
K08 = Clinical Investigator Awards
P30 = Core Centers
P60 = Comprehensive Centers
N01 = Contract Proposals
Other = Cooperative Agreements and Cooperative Clinical Studies

A request for the establishment of two standing review groups for handling manpower applications is pending. These will be in addition to the chartered Special Project Review Committee which reviews Multipurpose Arthritis Center applications.

The Review Branch is comprised of two sections with a total of twelve people. Of these, six are Executive Secretaries responsible for the selection of reviewers and the conduct of the technical merit reviews for the broad spectrum of applications received by the NIADDK. One of the more significant accomplishments this year has been the rescheduling of the receipt dates for competing continuation Multipurpose Arthritis Center applications. This rescheduling has alleviated the concentration of such applications at any one particular council round, distributing them more evenly throughout the year.

The major problem affecting the ability of the Review Branch to perform its mission is the departure of the Chief of the Review Branch. The Chief of the Review Branch provided the review expertise, direction, and guidance needed to assure timely and quality merit review. The Associate Director, DEA, has been serving as Acting Chief during the interim until a replacement is found.

A centralized "working review" file system needs to be created to enhance our review activities, in terms of both quality and more effective use of staff. Implementation is pending with the selection of a new Review Branch Chief.

ANNUAL REPORT

THE DIVISION OF

ARTHRITIS, MUSCULOSKELETAL AND SKIN DISEASES

ARTHRITIS RESEARCH AND TRAINING PROGRAMS

Overview

The content of the research effort in arthritis can be considered in four general areas that consume roughly equal portions of the program budget. The first of these includes the projects associated with immune disturbances, autoimmune diseases, rheumatoid factor, antigen-antibody reactions and immune complex formation. The second area consists of research concerned with collagen, connective tissue, basement membrane, synovial fluid and articular cartilage. The third area consists of research projects in rheumatic diseases; rheumatoid arthritis, degenerative joint disease, systemic lupus erythematosus, gout, heritable disorders, and many others. The fourth area concerns muscle research associated with the structure, function, contraction, and metabolism of skeletal muscle.

Program Activities and Accomplishments

The Program continues to be separated organizationally as Arthritis 1A and Arthritis 1B to give visibility to rheumatic disease activity and skeletal muscle research as distinct entities. Annual data summaries now display the total number of projects and dollars obligated for each of these major components of the program.

Arthritis 1A: The regular research category is comprised of 171 projects that total $19,726,087. Of this total number, regular research projects are 155, program projects are 8, and new investigator research awards are 8. The "other" research category is comprised of 18 projects that total $1,346,333; fifteen of these are career awards, there is one core center grant, one research and demonstration grant and one conference grant. Training consists of 28 institutional training programs and 7 individual fellowship grants totaling $1,591,137. The total support for all mechanisms is $22,663,557 for 224 projects.

Arthritis 1B: The regular research category consists of 59 projects that total $5,189,085. Of this total number, 56 are regular research grants and 3 are new investigator research awards. The "other" research category is comprised of 9 career awards only for $337,839 and the training effort is confined to 5 individual fellowships for $111,892. There are no institutional training awards for this part of the arthritis program which is concerned with skeletal muscle research. The total support for all mechanisms is $5,638,816 for 73 projects.

A change in content of the skeletal muscle component of the program is continuing over an extended period of time and is being achieved by assigning certain new and renewal projects to the Musculoskeletal Diseases Program. Projects that are concerned with skeletal muscle that relate to exercise physiology, locomotion, kinesiology, and atrophy and hypertrophy are being assigned to the Musculoskeletal Diseases Program consistent with the more broadly defined objectives of that program. Gradual reassignment prevents large, rapid dislocations of funds between the programs and the program management difficulties associated with such dislocations

Support was provided for the 1981 Gordon Conference on Structural Macromolecules - Collagen. This meeting was an outstanding international conference where the exchange of recent information in a rapidly expanding field was discussed by participants from the fields of molecular biology, cell biology, and physical chemistry. Characterization of the family of collagen genes, transcription and mRNA translation have recently become a very active research field and investigators with background in the molecular biology of nucleic acids have turned to analyses of the collagen genes and their expression. Since the structural studies of the collagen molecules are advancing rapidly, this meeting, where protein biochemistry, biosynthesis and studies of the collagen genes and their expression could be evaluated together, was of great importance and the information obtained had much broader significance.

Collagen-Induced Experimental Model of Arthritis

An experimental model of arthritis has been produced in approximately 40 percent of rats by immunization with native type II collagen and incomplete Freund's adjuvant. Success has been achieved in demonstrating the unique specificity of this model with respect to the nature of the antigen and documentation for both the cellular and humoral immune responses to type II collagen in rats with this type of experimental arthritis as well as in patients with rheumatoid arthritis and psoriatic arthritis. It has been shown that type II collagen-induced arthritis in rats can be transferred passively with syngeneic spleen and lymph node cells and that the disease can be modulated by stress. It is important to determine if this autoimmune response of the rodent immune system to collagen has general applicability.

Further work with this model will determine the immunologic mechanisms involved in the pathogenesis of this collagen-induced arthritis and to assess the relative roles of T- and B-cell responses to collagen in this system. The hope is to determine whether both humoral and cellular responses to collagen are required to produce arthritis and whether T-cell responses to native and denatured type II collagen in rats are under genetic control as they appear to be in mice and man. Additional studies are planned to answer the following questions: a) What is the sequence of pathologic events in collagen-induced arthritis in rats? b) Do neuroendocrine mechanisms (glucocorticoids, chtecholamines) explain how stress modalities modulate collagen-induced arthritis? c) Are cellular responses to other collagen types a feature of this type of collagen-induced arthritis? d) Do rheumatoid factors develop in mice with collagen-induced arthritis? e) Is there a temporal association between the acquisition of humoral or cellular sensitivity to collagen and the onset of collagen-induced arthritis in mice?

Role of Immune Complexes in Rheumatoid Arthritis

Another fairly large body of experience has emphasized the importance of the Immunoglobulin G-Rheumatoid Factor (IgG-RF) complex in the pathogenesis of both rheumatoid synovitis and the systemic complications of rheumatoid arthritis. Evidence has accumulated that the serum of certain patients with rheumatoid arthritis contains IgG-RF complexes of intermediate size and, although present in the serum of some patients with uncomplicated classical rheumatoid arthritis, these complexes are more commonly found in the serum of patients with systemic complications. Many of the tissue reactions entered into by the IgG-RF complexes are also entered into by IgM-RF to form larger immune complexes. These complexes, which may be phagocytosed by synovial lining cells and polymorphonuclear cells, are associated primarily with the formation and release of lysosomal enzymes. By virtue of these reactions and secondary reactions which follow, IgG-RF complexes appear to initiate the immune response of rheumatoid synovitis.

Inhibition by Retinoic Acid of Collagenase Production in Rheumatoid Synovial Cells

Researchers at Dartmouth Medical School are investigating the effects of retinoic acid, a vitamin A derivative, on cultured synovial lining cells from patients with rheumatoid arthritis (RA). They have shown that retinoic acid completely inhibits production of collagenase (which destroys cartilage) and moderately inhibits production of prostaglandin E-2 (which fosters inflammation and retards cartilage repair). Thus, retinoic acid has beneficial properties comparable to those of corticosteroids, but may not have the undesirable side effects of steroids. Clinic trials with retinoic acid in RA are in order.

Involvement of Prostaglandins in the Course of Rheumatoid Disease

The prostaglandins, long-chain fatty acids, produced by the loose layer of connective tissue (synovial membrane) lining the joint cavity are believed to modulate the inflammation of rheumatoid arthritis possibly by inhibiting the formation of new cartilage that could replace that destroyed by protein-degrading enzymes. To test this hypothesis, slices of animal joint cartilage have been incubated in various prostaglandin-containing media, including that derived from normal and rheumatoid human joint fluid. The findings suggest that prostaglandins present in human rheumatoid joint fluid significantly inhibit the metabolism of animal cartilage in culture. The precise role of prostaglandins in the cartilage destruction experienced by rheumatoid arthritis patients still remains uncertain. Prostaglandins, therefore, have been shown to participate in the cartilage destruction of patients with rheumatoid arthritis; the detailed mechanisms by which this takes place need to be elaborated.

Role of the Humoral Immune Response in the Pathogenesis of Systemic Lupus Erythematosus

The humoral immune response to DNA has been strongly implicated in the induction of nephritis, the major cause of death in systemic lupus erythematosus. It appears likely that only some subpopulations of antibodies to DNA are pathogenic, since it was shown earlier by others that normal individuals can produce antibodies to DNA. Data showing restricted heterogeneity of anti-DNA antibodies in the glomerule of mice with lupus suggest this hypothesis is correct. Success has been achieved in the production of several different monoclonal murine antibodies to ssDNA and dsDNA making it possible for the first time to study antigenic specificities and pathogenic properties of each subpopulation. The animal data will provide new information concerning disease pathogenesis, especially if similar results are forthcoming in human studies. A new therapeutic concept will result, if suppression of pathogenic subpopulations with anti-idiotypic sera is successful in preventing nephritis without suppressing normal immune responses. An additional benefit from these studies is that development of monoclonal antibodies to DNA which recognize specific base sequences will provide probes for the study of RNA and DNA molecules.

Selective Suppression of Collagen Synthesis by Anti-Inflammatory Steroids

Previous studies have suggested an apparent selective suppression of collagen synthesis in connective tissue upon treatment with glucocorticoids in intact animals and clear implications now exist for a better understanding of fibrotic processes and the problems that arise from the use of glucocorticoids as anti-inflammatory agents. Further, it has been shown that the same degree of selective reduction in collagen occurred at least in two human cell lines as in the animal study. Additional work will help to elucidate gene expression in normal fibroblasts and in those treated with anti-inflammatory steroids at the nucleic acid level and to determine the molecular basis of this selective suppression of collagen synthesis. Separate studies have been initiated to examine the effects of glucocorticoids on the synthesis and degradation of type I and type III collagen because a differential influence on the ratio of type I to type III was observed. Since glucocorticoids are a standard form of anti-inflammatory therapy, such studies could have far-reaching clinical implications by providing a rationale for their use that is less empirical and more mechanistic.

Discovery of Inflammation-Inducing Factor in Gout and Pseudogout

A significant contribution has been made to knowledge of the pathogenesis of the acute highly inflammatory crystalline-induced arthritis of gout and pseudogout. Investigators at Washington University have shown that when polymorphonuclear (PMN) leucocytes ingest either urate or calcium pyrophosphate crystals, they (the PMNs) release a factor, called crystal chemotactic factor or CCF. This factor has been shown, when injected into rabbits, to induce an inflammation indistinguishable from that seen in crystalline-induced acute arthritis.

Factors Controlling the Self-Association of Certain Immunoglobulins

There are continuing needs to explore the link between rheumatoid arthritis and one or more defects in immune mechanisms. Much is yet to be learned about various immunoglobulins (complexes made up largely of immune antibodies) by measuring the physical characteristics and biological activity of these complexes. A specific and sensitive assay method has recently been developed for performing detailed laboratory analyses of immunoglobulins. Moreover, these researchers have discovered a self-association phenomenon in certain immunoglobulins, leading to formation of immune complexes that contain only antibody molecules (instead of aggregation of combined antigen-antibody molecules). Future work will focus on finding the determinants of the self-association process, which - if found to be common in rheumatoid arthritis patients - may identify an important element which maintains inflammation in joint membranes.

Human Immune Regulation in Immunodeficiency and Connective Tissue Diseases

A systematic approach to the study of human disease states is being continued which is highly relevant to current issues of human immune regulation. The ongoing work is directed at extending insight into abnormalities of immune regulation, particularly in patients with immune deficiencies, rheumatoid arthritis, systemic lupus erythematosus, chronic active liver diseases and other connective tissue disorders. The study of T cell help or suppression, the relative contributions of suppressor T cells, the effects of soluble suppressor factors elaborated by cultured T and B cells, the effects of thymic hormone preparations, and the analysis and identification of alterations in various T cell subsets in normal individuals and patients with immune deficiency and connective tissue disorders will allow new observations which may have immediate clinical significance.

Characterization of Nuclear Antigens Reactive with Antibodies from Patients With Mixed Connective Tissue Diseases and Scleroderma

This study will continue to determine structure, function, and immunologic properties of nuclear macromolecules which are the targets of autoimmune antibodies in the rheumatic diseases. The focus is on two antigens: a class of nuclear RNA-protein complex and a basic chromosomal protein (Scl-70). Their corresponding antibodies occur in MCTD and scleroderma, respectively. The core of the antigen is composed of a protein and RNA but the function of the RNA remains unknown and the RNA of the core antigen will be analyzed and compared to other nuclear RNA classes to determine the extent of homology or complementarity. A scleroderma-associated antigen, Scl-70, has been isolated and identified and its primary and higher order structure will be investigated and coordinated with efforts to identify its important antigenic features. Other objectives are to identify the preferred substrates for the enzyme and to determine the immunoglobulin subclass composition of anti-Scl-70 antibodies. A good start has already been made on the studies proposed.

Immune Disturbances in Rheumatic Disease

The prevalence of self-associating IgG-rheumatoid factors are being investigated in various forms of rheumatoid arthritis and in other conditions to assist in assigning these immune complexes a pathogenic role in rheumatoid arthritis. Detailed studies of synovial fluids are examining the possible involvement of other antigen-antibody systems. The results of these investigations may determine the relative role of self-associating IgG-rheumatoid factors and other possible antigen-antibody systems in rheumatoid synovitis. This information will be of significance in directing further inquiry to a single or multiple antigen-antibody systems to elucidate the events or factors that cause rheumatoid arthritis. Studies are examining how the self-associating IgG-rheumatoid factors interface with known pathways of inflammation.

Infectious Arthritis: Mycoplasma

The mycoplasmatales are ubiquitous prokaryotic organisms causing disease in animals and man as well as in plants. Interest in these organisms centers primarily upon their arthritogenic potential. It is noteworthy that nine different species of mycoplasmas are capable of inducing chronic arthritis in animals. Although it has been suggested that these organisms might be involved in human rheumatoid arthritis (RA), a number of seemingly important reports have not been confirmed.

There is increasing evidence, however, that mycoplasmas may cause nonrheumatoid arthropathies of man particularly in immunocompromised individuals. Despite the negative studies to implicate mycoplasmas in RA, mycoplasma-induced arthritis of animals closely resembles RA in respect to both course of disease and histopathology with a typical, chronic inflammatory pattern with synovial membrane hypertrophy, pannus formation and cartilage and bone erosion.

Some mycoplasma are mitogenic for the lymphocytes of mice and humans, but minimally so for rabbit lymphocytes. *M. arthritidis* proved to be primarily a T cell mitogen but require helper macrophages for activation. *M. arthritidis* also stimulate lymphocytes to cytoxic activity; this stimulation did not appear to require monocytes. Studies of chronic arthritis in rabbits demonstrated that arthritis continued, despite the disappearance of the mycoplasma by seven weeks following intraarticular injection. Immune complex deposition in tissues and cartilage, elevated levels of anti-mycoplasma antibody in joint fluid, and erosion of cartilage have all been observed.

Rheumatoid Arthritis: Role of EB Virus

The association of a previously unrecognized precipitating antibody (RAP) in the serum of patients with rheumatoid arthritis (RA) that is specifically reactive with a nuclear antigen (RANA) from cells transformed by the Epstein-Barr virus (EBV) raised the possibility that the virus might play an etiologic role in this disease. Subsequently, it has been convincingly demonstrated that the RAP and/or anti-RANA antibodies only indicate prior EBV exposure, and they are not unique to RA. RA patients, however, do have greater amounts of these antibodies, as well as higher titers of antibodies to a variety of other EBV related antigens when compared to controls and cultured lymphoid cells from these patients show an increased frequency of spontaneous lymphoblast transformation and a more rapid rate of EBV-induced lymphoblast transformation following *in vitro* infection. These findings suggest impaired regulation of EBV virus infection by RA mononuclear cells.

Lymphoid cells from patients with rheumatoid arthritis (RA) are impaired in their ability to control Epstein-Barr virus (EBV) infection. EBV-induced lymphoblast transformation following *in vitro* infection occurs more rapidly in RA cells, and spontaneous lymphoblast transformation occurs more frequently in cells obtained from the blood of RA patients than it does in cells from healthy individuals. The regulation of EBV-induced lymphoblast proliferation can be divided into "early" and "late" phases which appear to be mediated by cellular events and by soluble factors derived from activated cells. The plan is to continue the dissection of these regulatory events, comparing the function of cells from healthy individuals with those from RA patients. Studies of the direct cell-mediated events will be directed at phenotypically characterizing the responsible regulatory subpopulations and determining if the regulatory defects in the RA cells is specific for EBV or a more generalized defect in the ability to control B cell proliferation. Studies of the soluble regulatory factor will include analysis of the interacting cell populations required for its generation; analysis of the role of prostaglandins in the diminished capacity of RA cells to generate it; and analysis of the soluble factor as an interferon.

Systemic Lupus Erythematosus: Immunologic Studies

Monoclonal antibodies to DNA derived from MRL/1 mice react with the sugar phosphate "backbone" of DNA and by that reactivity are also capable of reacting with similar structures in phospholipids. Other antigenic specificities are present in some clones, including recognition of nucleosides. The production of heterologous anti-idiotypic antisera in rabbits resulted in one anti-idiotypic antibody (AIA), which reacted with the majority of anti-DNA antibodies in sera of MRL/1 mice, including some antibodies which did not have reactivity with DNA. Such anti-idiotypic antibodies might have influences on multiple anto-antibodies reacting with nucleic acids, cell surfaces, histone, etc., in both murine and human SLE. These may foster innovative studies of disease pathogenesis, and even possible therapeutic interventions. Monoclonal DNA antibodies from fusion of SLE human B cells with the two secreting human myeloma cell lines U266 (secreting IgE) and GM1500 (secreting IgG2) are being produced.

Careful studies of anti-DNA antibodies in patients with SLE have shown the presence of high and low affinity antibodies and their ratios in various SLE sera, *in vitro* formation of very large, soluble complexes in antibody excess with the high affinity species, and their capacity to activate complement. These complement-activating complexes are not detected by a Clq-binding assay. Cryoprecipitates formed with anti-DNA antibodies in SLE appear to contain antibody in great molar excess relative to antigen. In addition, DNA of sufficient size is necessary to form immune complexes capable of activating complement.

The humoral immune response to DNA has been strongly implicated in the induction of nephritis, the major cause of death in systemic lupus erythematosus (SLE). It appears likely that only some subpopulations of antibodies to DNA are pathogenic, since it was shown earlier by others that normal individuals can produce antibodies in the glomeruli of mice with lupus suggest this hypothesis is correct. Success has been achieved in the production of several different monoclonal murine antibodies to ssDNA and dsDNA making it possible for the first time to study antigenic specificities and pathogenic properties of each subpopulation.

Accomplishments include the following: (1) Demonstration of suppression of both ssDNA and dsDNA antibodies by induction of immune tolerance to ssDNA and the subsequent prevention of nephritis in mice with SLE; (2) demonstration of restricted heterogeneity of DNA antibodies eluted from glomeruli compared to serum antibodies in MRL/ℓ, BXSB and NZB/NZW f_1 mice; (3) production of monoclonal antibodies to dsDNA from MRL/ℓ, BXSB and NZB/NZW f_1 spleen cells similar in pI to subpopulations in renal lesion; and (4) production of an idiotypic antibody against one monoclonal dsDNA antibody.

Experimental Myotonia

Myotonia is characterized by a prolonged involuntary contraction of muscles lasting several seconds following a voluntary contraction or in response to direct muscle stimulation. It can be defined in electrophysiological terms as an abnormal tendency of the muscle membrane to discharge repetitively upon single stimulation. This hyperexcitability persists even after nerve block, denervation, or curarization. It may be exacerbated by exposure to cold and tends to lessen with exercise. Several different diseases have been described which have in common the muscle myotonia as a prominent clinical finding. Among these, three inherited diseases have been identified in which myotonia is a major clinical manifestation. These are: (1) myotonia congenita, (2) paramyotonia congenita, and (3) dystrophia myotonica.

Studies of the properties of ionic channels in experimental myotonic muscle (both dystrophic and congenital models), with the aim of more firmly establishing the cellular basis of the clinical manifestations of myotonia are in progress. Techniques were developed and preliminary observations have been conducted establishing the feasibility of the proposed experiments. In preliminary studies relevant to the proposed work, the Hille-Campbell vaseline seal clamp has been set up and voltage clamp current records have been successfully obtained from single rabbit extensor longus digitorum (EDL) muscle fibers. For the sodium channel, preliminary observations have been made on current-time and current-voltage relations, h_∞ curves, and the effects of tetrodotoxin and temperature have also been examined. In addition, preliminary voltage clamp studies on the effects of two agents, 9-anthroic acid (9-AC) and 2, 4-dichlorophenoxyacetic acid (2, 4-D), which induce myotonic behavior have been conducted. No effects on Na channel behavior were found that would account for the induced repetitive discharges. Finally diphenylhydantoin (DPH) which has anti-myotonic action was shown to suppress the repetitive discharges produced by 9-AC.

MUSCULOSKELETAL DISEASES RESEARCH AND TRAINING PROGRAMS

The Musculoskeletal Diseases Program provides research support for basic and clinical studies on the various components of the musculoskeletal system in normal and diseased conditions. The program utilizes funding mechanisms such as regular research grants, career development awards, training grants and contracts. The total budget for these activities was approximately $14.5 million in FY-82, supporting nearly 200 active grants.

The total training and manpower support has remained level during the past year. Institutional training grants are constant at ten. Individual fellowships have decreased slightly, while career development awards are somewhat increased.

The Program and its staff have participated in and supported several important conferences and workshops during FY-82. An NIH Consensus Development Conference on Total Hip Joint Replacement was held in the Spring of 1982. Oral presentations and extended abstracts were provided to a panel of experts and public representatives. The panel was charged with preparing a document assessing the state of knowledge regarding this surgical procedure. The overall success of hip joint replacements was documented with reports of 90 percent positive results 10 years after implantation. The panel report is being widely disseminated, including publication in the Journal of the American Medical Association.

A workshop was convened to discuss sports medicine research with special emphasis on the knee. This interdisciplinary meeting included scientists from the fields of orthopedics, biomechanics and biochemistry. Each structural component of the knee was discussed in terms of basic properties, injury phenomena, diagnosis and treatment. The participants identified several research areas requiring future investigations utilizing multidisciplinary teams.

The Program partially supported the second Gordon Conference on Bioengineering and Orthopedic Science. Each day of the meeting was devoted to a specific area such as muscle mechanics, lumbar spine, bone response to implants, and connective tissue response to stress. The informal discussions lead to a free exchange of new and creative ideas.

A major project to evaluate the Musculoskeletal Program has been in progress for over one year. Four task group panels are assessing the scientific progress and program management during the period 1975 to 1980. A large volume of summary data was provided to the panelists. Forms to assist the evaluation process were developed by the contractor and NIADDK staff and utilized by the evaluators. The next step will be to provide the Program with scientific direction and management guidelines for the future.

Basic Bone Research

Advancements in clinical medicine require a solid foundation of continued support for basic research. The Musculoskeletal Diseases Program funds projects on the growth, structure, metabolism, and mineralization in normal and abnormal bone.

Vitamin D and its metabolites play a major role in the absorption of calcium and subsequent mineralization of bone. Researchers at the University of Arizona have identified target cells for 1,25 $(OH)_2$ Vitamin D in many organs, including bone. In certain situations this receptor has been found to be absent. Investigators have shown that estrogen affects the sensitivity of the receptors; however, the rate is variable from organ to organ. The overall significance of this Vitamin D metabolite and the function of the receptors are currently unclear. Studies at the Washington University (St. Louis) could demonstrate only a direct influence of 1,25 $(OH)_2$ Vitamin D in bone tissue. Another researcher found a simple control system that could explain the regulation of hydroxylase conversion of 25 (OH) Vitamin D into its further metabolites. At the University of Pennsylvania scientists have found actin to be a Vitamin D binding protein. They also discovered that 25 (OH) Vitamin D stimulates muscle metabolism.

The epiphyseal growth plate plays an important role in the development of the maturing skeletal system. Investigators at Montefiore Hospital have observed in the growth plate proteoglycans with huge aggregate size as compared to mature cartilage. Proteoglycan monomer and link proteins persist without significant loss or degradation during mineralization. This result refutes the theory that mineralization requires protoglycan degradation. Another scientist has shown a stable "resistant" proteoglycan with variable distribution in the growth plate but constant presence in calcified cartilage. At the University of Pennsylvania researchers found calcium flux leaving the mitochondria at the same rate as matrix vesicles gain calcium. Whereas these investigators have had little success in electrically stimulating growth plates _in vivo_, there have been recent positive results _in vitro_ using capacitive coupling.

The specific mechanisms of endochondral mineralization are the subject of intense scientific investigations and controversy. At Pennsylvania State University mineralization was found to occur first in matrix vesicles which enlarge as they acquire mineral. They noted an early mix of amorphous and crystalline structures. Scientists at the University of South Carolina showed that matrix vesicles were formed from cellular products and not dead cells. These investigators also defined more clearly the role of alkaline phosphatase and established the passive nature of uptake for mineral ions. In contrast, scientists at the Children's Hospital (Boston) using X-ray radial distribution technology did not observe any significant amorphous calcium phosphate in growth plates. Phosphoprotein content had the largest increase in areas of initiation of mineralization.

Once formed, bone remodels in response to its mechanical loading environment. At Tulane University, researchers have developed a model that predicts the inward or outward restructuring of periosteal and endosteal bone surfaces in response to variable axial loads. Experiments indicate that periosteal changes dominate. Another study showed a greater increase in remodeling with bending than with axial loading. In a model developed at Beth Israel Hospital (Boston) struts of cancellous bone are believed to be oriented to minimize bending on each tiny piece of bone.

Bone Diseases

Many of the projects within the Musculoskeletal Diseases Progam are directed towards specific bone disease, including both acquired and hereditary disorders.

Osteoporosis is a major medical condition affecting millions of post menopausal women. In response to a Program initiative, two projects were funded to conduct a clinical trial on fluoride therapy. The two centers at Mayo Clinic and Henry Ford Hospital plan to recruit a combined total of 200 patients. Much of the effort in the first year has been to locate, pre-test, and stabilize the treatment of the prospective patients. A common protocol has been developed for use at both centers. The two-drug protocols are: 1) calcium and fluoride and 2) calcium and placebo.

Epidemiologic data developed at the Mayo Clinic indicate an economic cost of $1 billion annually for treatment and care of hip fracture victims in the nation. The study showed that the rate of bone loss was different in weight bearing and non-weight bearing bones. Spinal bone loss is linear with age. In osteoporosis, the loss of bone is accelerated in the axial skeleton. Using CT scans, researchers at University of California, San Francisco define "normal" post-menopausal bone loss as 1.7% per year. However, in osteoporotics the cancellous bone loss is 9% per year and cortical bone loss is 2-3% per year. Other studies confirm the larger loss of cancellous bone from endosteal surfaces. A study has just been initiated at University of Pittsburgh to define the correlation between physical activity and bone mass in post-menopausal women. A new highly sensitive CT scanner has been developed for use in this study.

Among other clinical studies using small groups of patients at Creighton University, calcium balance was shown to relate directly with calcium intake. This is the basis for the typical recommendation for calcium supplementation. Estrogen increase serves to increase the calcium baseline. In general calcium, estrogen, and fluoride all have been shown effective in small sample sizes and uncontrolled studies. In fact, a recent Mayo Clinic report shows the results to be cumulative; a combination of all three works best.

Several studies concentrate on inherited disorders of connective tissue. Osteogenesis imperfecta (OI) is manifested in many forms. At Johns Hopkins University, investigators observed two dominant forms with and without opalescence of teeth. The opalescent form has a normal ratio of Type III to Type I collagen. The researchers identified and catalogued a variety of secondary conditions resulting from this disorder. Biochemical studies at two universities have shown several defects in collagen synthesis and processing. In some types of OI there are cross-linking errors, whereas in other types the collagen is normal but reduced. At University of Connecticut, scientists are using newly developed DNA probes to look at RNA for pro-alpha I and pro-alpha II chains. Preliminary results point to unstable helix formation and improper balance between Type I and Type II chains.

Osteoarthritis

Osteoarthritis is a major disabling disease in which the cartilage degenerates progressively. At Massachusetts General Hospital, researchers have shown a marked increase in rates of synthesis for proteoglycans, collagen, and hyaluronic acid as early events in the degenerative process. These responses do not seem to prevent tissue destruction. Recent this laboratory observed chondrocyte heterogeneity in joint cartilage, uniformity of link protein in normal and disease states, and identified a high molecular weight protein occurring only in osteoarthritic cartilage.

At the Children's Hospital (Boston), a rabbit model with injury-induced arthritis demonstrated an early increase and subsequent decrease in cartilage water content and a large initial decrease in uronic acid followed by a return to normal. Other researchers have identified a role for lysozyme in disaggregating the large proteoglycan complexes. Using tissue samples, scientists at Tufts University documented that osteoarthritic human cartilage produces Type II collagen as in normal cartilage rather than "abnormal" Type I collagen as previously reported. Vitamin C and Vitamin E both modulate the biosynthesis of sulfated proteoglycans in human cartilage. Studies in an animal model confirmed that high levels of Vitamin C reduced significantly the severity of the degenerative process.

A study on mechanical failure in osteoarthritis found a decrease in shear modulus in areas adjacent to cartilage lesions. The compression modulus seemed to be related to the biochemical composition (uronic acid concentration). Researchers at Rensselaer Polytechnic Institute are comparing the mechanics, structure, and chemistry of normal and abnormal cartilage in osteoarthritis. Recent findings include: tissue swelling and shrinkage are directly related to salt concentration; shear modulus depends on frequency and strain, shear modulus is proportional to collagen content; elecrical streaming potential is much more significant than piezoelectric effects; and fluid flow is important over a wide frequency range.

Spinal Disorders

Scoliosis appears to be readily controllable when detected early. Investigators at Fox Chase Cancer Center have shown a strong correlation between early changes in visual vestibular ocular behavior and the degree of spinal curvature. Results demonstrate the ability to predict a high risk of future curvatures in the siblings of scoliosis victims. At the University of Vermont scientists are evaluating the accuracy of non-invasive techniques for screening of spinal curvature. The Moire fringe method measures the distortion of a square grid pattern projected on the back. In another study, a balance force plate device was able to differentiate between progressive and non-progressive types of scoliosis.

At George Washington University researchers found significant alterations in the types of collagen in the intervertebral disc of persons with scoliosis. Increases in Type 1 collagen in disc annuli were linked to the duration of the curvature and is probably a result rather than a cause of the curvature. In a genetic experiment with a chicken model, scoliosis was associated with three major autosomal recessive genes. Biomechanical studies at Case Western Reserve noted that scoliotic patients had statistically significant increase in intervertebral disc height at the apex of each curve as well as abnormal anterior-posterior wedging.

A major workshop to stimulate research on the etiology of low back pain was held the previous year and its proceedings are being published. Some new projects on low-back pain are now being supported, perhaps as a result of this effort. Biochemical studies are in progress to characterize normal and degenerative discs. At the University of Minnesota, proteogylcan from human nucleus pulposis was found in many forms ranging from small fragments to large aggregates. Utilizing a rabbit model, investigators unexpectedly found no significant difference between facet removal and a sham operation. Initial results from chymopapain studies indicate a potential for partial recovery.

Clinical stability of the functional spinal unit has been studied at the Beth Israel Hospital (Boston). Proposed guidelines suggest that 2.5 mm of horizontal translation in the sagittal plane and/or 5 degrees of rotation in that same plane will result in instability. The investigators also measured differences in the mechanical properties for each of the lumbar segments. Studies have been initiated to evaluate the biomechanical and biomechemical changes from spinal injuries and from subsequent surgical repair.

Exercise Pathophysiology

Ligaments and tendons are specialized connective tissues often injured in sports and occupational activities. Two studies are measuring the effects of joint immobilization on these connective tissues.

At the University of California, San Diego scientists demonstrated the early onset of atrophy in connective tissue (10-12 weeks) and the lengthy time for compensatory hypertrophy (12 months). They also suggested potentially therapeutic measures to minimize the observed changes of molecular cross-linking.

At the University of Cincinnati, a study has shown an inverse relationship between the load applied and blood flow in tendons. The investigators quantitated three-dimensional kinematics of a clinical examination for anterolateral rotatory joint instability. Few of the current procedures that incorporate tendon and ligament transfers are structurally capable of supporting the anticipated loads. At the University of Vermont, a new micro-strain transducer has provided clinically relevant data on levels of safe loading of ligaments during clinical testing and convalescent conditions such as casting and bracing.

Several studies of muscle activity and alteration during exercise and disuse are in progress . During exhaustive exercise rates of muscle protein synthesis decreases and rates of protein degradation are increase. According to most investigators, exercise training can increase the size of existing muscle fibers. An investigator at University of Texas, Dallas has demonstrated a mechanism for fiber splitting that adds new fibers to the muscle mass. Other studies at State University of New York have classified muscle tissues in terms of oxidative capacity. Lower oxygen capacity supports low rates of contraction without fatigue. Training will induce a functional advantage by delaying fatigue and sustaining ATP provision. Slow muscles recover from fatigue in less time than fast muscles. Another study has demonstrated distinct anatomic distribution patterns for oxidative and non-oxidative muscle types.

One study has shown similar patterns of muscle fiber recruitment in uphill and downhill racing; however, more damage to muscle fiber was observed in downhill racing . Another functional study has demonstrated that the interaction between discrete parts of a single muscle must be evaluated to obtain meaningful data. The spatial order of muscle fiber recruitment was found to be the same as force was increased, whether by increasing speed, running uphill or carrying loads.

At Marquette University disuse (decreased motor activity) affected all muscle fiber types in specific, and often different ways. For example, disuse shortens both contraction time and relaxation time in the slow soleus muscle while prolonging these contractile properties in fast muscle. Another researcher established the time course of loss of protein and wet weight in skeletal muscles of immobilized rat limbs. One week after starting the disuse program. The muscle had atrophied by half. Slow twitch muscle recovers at a faster rate than does fast muscle.

SKIN DISEASES RESEARCH AND TRAINING PROGRAMS

The Skin Diseases Program of the NIADDK provides research support for basic and clinical studies of the skin in normal and diseased states. Areas of emphasis include: psoriasis and disorders of keratinizing tissues; vitiligo and other disorders of pigmentation; photobiology, photo-allergy, and phototoxic reactions; metabolic studies of skin, including effects of hormones and interaction with enzymes; immunologically mediated cutaneous disorders, including atopic dermatitis, eczema, contact dermatitis, and vasculitis; bullous diseases of the skin, including pemphigus, pemphigoid, dermatitis herpetiformis, and epidermolysis bullosa; acne and physiologic activity of the sebaceous gland; disorders of hair growth, including alopecia areata; and cutaneous manifestations of connective tissue disease disorders, including lupus erythematosus, scleroderma, and pseudoxanthoma elasticum.

The Skin Diseases Program utilizes many funding mechanisms, including research grants, career development awards, Clinical Investigator Awards, New Investigator Research Awards, training grants, and contracts.

The Skin Diseases Program contributed significant support to the 31st Annual Symposium on the Biology of Skin, on the subject of "Structural Elements of the Dermis." Among topics discussed were: transmission and scanning electron microscopy of developing dermis; physical properties of the dermis as well as its unique association with epidermis; genetic heterogeneity of collagens; the biosynthesis, structure, and function of the carbohydrates, link and core proteins of proteoglycans, as well as proteoglycan storage diseases; the etiology, pathogenic mechanisms, and molecular defects in scleroderma and Ehlers-Danlos syndromes; and some new findings in osteogenesis imperfecta and epidermolysis bullosa. The proceedings of this symposium will be published in the Journal of Investigative Dermatology later this year.

The Skin Diseases Program also supported in part a workshop on Oral Retinoids in Dermatology which discussed the mechanism of action of these agents, their effect on sebaceous glands and acne, their pharmacology and toxicology, their use in keratinizing disorders, and their potential use in tumor prevention. The proceedings of this workshop was published in the April 1982 issue of the Journal of the American Academy of Dermatology.

Keratinization

Much recent work in the field of keratinization and keratin structure has utilized gene mapping and messenger RNA - DNA hybridization techniques to investigate the basic structure of the various epidermal proteins, and particularly the filamentous proteins including keratin. In addition, there have been advances in the investigation of keratinocytes, their metabolic products, and the stratum corneum permeability barrier.

Workers at Boston University have demonstrated that during normal keratinization, similar soluble proteins are retained in the horny layer regardless of body region. However, during pathologic keratinization, such as in psoriasis, different soluble proteins are retained in the scales. Studies at the Massachusetts General Hospital indicate that the fibrous protein of hair and epidermis are consistently different in composition. They have also shown that stratum corneum fibrous proteins of psoriatic plaques are abnormal as compared to those of normal stratum corneum but that these proteins revert to normal after successful therapy for psoriasis. However, this return to normal does not occur until after the skin appears normal clinically.

Other studies have focused on specific factors in the keratinization process. Investigators at the University of California, San Francisco, have discovered that histidine-rich proteins in epidermis appear in different forms: a diffuse form observed in 10-12-day old fetuses, and granular forms which may be in fetal-type granules and keratohyalin granules. Since fetal-type granules are not involved in the formation of cornified cells, this indicates that the function of histidine-rich proteins is not limited to the formation of the matrix in cornified cells. According to studies at Northwestern University, lamellar granules contain phospholipids, sterols, sphingolipids and hydrolytic enzymes capable of breaking down these lipids, and that they are packaged for extracellular transport.

Recently there has been a great increase in the use of hybridization techniques in the investigation of the genetics of keratin. In parallel studies of guinea pig keratin and mouse hair root, investigators at New York University have been able to extract messenger RNA and use this as a template for hybridization with complementary DNA which could then be identified and cloned using plasmids in E. coli. Utilizing this DNA, the amino acid sequence of this keratin may be determined. The post-translational modification of mammalian keratins has also been studied, focusing on the phosphorylation of two major phosphorylated keratin polypeptides, termed MEK 1 and MEK 2. These have been shown to be keratins through the use of immunofluorescent and immunoblot assays: the peptide map of these two proteins differ one from the other, and the proteins are phosphorylated at different sites. Although these molecules are both components of the intermediate filament cytoskeleton, they appear to subserve different functions within this structure. From research at the University of Chicago with bacterial plasmids containing complementary DNA sequences specific for keratins that were constructed from messenger RNA isolated from human epidermal cells, two major groups were identified which do not crosshybridize. These genomic fragments represent separate and probably complete keratin genes; and these two subfamilites of keratin genes are conserved through vertebrate evolution.

Several studies have investigated the role of epidermal lipids in the maintenance of the barrier function of the stratum corneum and defects in various genetic diseases. From research at the Veterans Administration Hospital in San Francisco, a disease-specific diagnostic accumulation of cholesterol sulfate was demonstrated in the stratum corneum of patients with x-linked ichthyosis. This finding provides the link between the known lack of the enzyme steroid sulfatase and abnormal scaling in this disease. They have also demonstrated an increase in n-alkanes in another recessive dermatosis, lamellar ichthyosis; this finding, however, is not disease-specific. Others have postulated that the enzyme steroid sulfatase causes enough desulfation of the putative stratum corneum glue, cholesterol sulfate, during stratum corneum maturation to permit normal shedding. These studies may prove important in the investigation of several of the ichthyoses.

Others at the University of Michigan have been investigating the effects of cyclic AMP, retinoids (particularly the clinically useful RO 10-9359 and 13-cis retinoic acid), TPA, arachidonic acid metabolites and mixed function oxygenases in epidermal cell function. The effect of retinoids on epidermal cell proliferation and differentiation in culture may suggest how this drug is beneficial in treating psoriasis. Epidermal cell differentiation experiments have shown that a precursor-product relationship between various epidermal cell differentiation proteins may exist. An imbalance in the system may be involved in skin diseases showing dyskeratinization. Workers at Cornell University are developing techniques for the tissue culture of epidermal cells without the presence of dermal fibroblasts. These cultures have been established as multilayered sheets with the cellular organization of epidermis in vivo. These studies are of great importance in establishing an in vitro model that will allow the investigation of multiple aspects of keratinocyte maturation and the process of keratinization.

Photobiology

A major research and clinical effort has been undertaken on psoralen plus ultraviolet A light (PUVA) therapy for psoriasis, its possible risks and long-term sequelae. In addition, there is continued interest and investigation into the porphyrias as well as into other more basic aspects of the interaction of light with various portions of the epidermis and dermis.

A long-term follow-up study of 1,380 patients who underwent PUVA therapy for their psoriasis five years ago is ongoing under contract. The risk of the development of squamous cell carcinoma is increased ninefold and seems to correlate with increasing exposure to PUVA. There is also an increased, but smaller, risk for the development of basal cell carcinomas and for keratoacanthomas. No ophthalmologic findings of significance have been reported. There are many intriguing questions in regard to the potential effects of PUVA, including questions of DNA-psoralen photochemistry, PUVA immunologic effects, and other carcinogenic effects of PUVA. Several of these have been addressed by individual grantees within the Skin Diseases Program. At the Massachusetts General Hospital studies indicate that long-term PUVA therapy has an immunosuppressive effect. They were based on examination of lymphocytes from PUVA-treated patients as well as guinea pig studies on immune function following PUVA and UVB irradiation. It was also noted that UVB irradiation is not without effect in these studies. These same researchers have also been investigating the optics of phototoxicity and have determined that UVA and UVB exposures were additive when the UVA exposure proceded the UVB exposure; they were less than additive when the UVB exposure preceded the UVA exposure. These and other similar studies have great potential for allowing us to select accurately light and chemicals for therapeutic effects while at the same time minimizing unwanted side effects.

A high pressure liquid chromatography technique has been developed at the University of Wisconsin that allows rapid, quantitative porphyrin analysis on small samples. Specimens from a large number of patients with different forms of porphyria are being examined to determine whether this technique will allow a more precise diagnosis in human porphyrias. At Columbia University patients with erythropoietic protoporphyria are being followed using various laboratory parameters in the hope that changes in these parameters will allow the prediction of those patients at greater risk for liver failure in this disease.

Workers at both the University of California, San Francisco, and at the Massachusetts General Hospital are investigating the mechanism of Vitamin D_3

production in skin and its control. Ultraviolet light of wave lengths between 295 and 300 nm. interacts with 7-dehydrocholesterol, probably in the basal layer of the epidermis. Moreover, the spectral character of natural sunlight modulates the cutaneous synthesis of previtamin D_3. Studies are ongoing to determine feedback control mechanisms that prevent vitamin D intoxication following large amounts of UV exposure. Workers at the Medical College of Wisconsin are investigating the interaction of free radicals with the pigmentary system in phototoxicity and have demonstrated that free radicals are produced both by melanin pigment in skin and by certain photosensitizing chemicals in response to light. These studies are relevant to understanding mechanisms of photodamage by sunlight both in the presence and absence of exogenous chemicals.

At Columbia University mutagenic and carcinogenic effects of ultraviolet radiation are being investigated in cell lines from patients with defects in the repair of UV-induced DNA damage. These studies may give us further insights into the mechanisms of UV-induced carcinogenesis and mutagenesis.

Immunologic Skin Disease

The clinical significance of the anticytoplasmic (Ro) antibodies in lupus erythematosus is being investigated at the Johns Hopkins University. Over 300 Ro positive patients have been examined; these patients may be subdivided into several groups clinically. Some are negative in testing for antinuclear antibodies, and have predominantly cutaneous lupus. Some have Sjögren's syndrome. Approximately 10-15% develop significant clinical renal disease. These investigators are also looking at anti-DNA antibodies, parameters of T cell function, and HLA antigens in an attempt to make valid predictions as to the clinical course of cutaneous lupus patients on the basis of these laboratory findings. Their work suggests the lack of a primary role for immunoglobulin, complement and specific autoantibodies in the pathogenesis of the cutaneous lupus lesions. Others at the University of Colorado have demonstrated that cellular cytotoxicity may be the key mechanism in the development of the lesions of cutaneous lupus. Workers at the University of Texas Health Sciences Center have confirmed these findings in patients with "subacute cutaneous lupus erythematosus." However, in patients with systemic lupus erythematosus, long term survival is significantly better in those patients without immune deposits, the so-called "lupus band," in normal skin.

Investigators at the University of Oregon Health Sciences Center are determining immune complexes in the serum of patients with allergic diseases of the skin, as well as mechanisms of tissue injury produced by these immune reactants. Sera from patients with erythema multiforme following herpes virus infections were found to contain immune complexes. However, these immune complexes were found not to contain intact herpes virus. Patients with dermatitis herpetiformis were found to have both IgA and IgG-containing immune complexes. In atopic dermatitis investigators are looking for evidence of staphylococcal antigens in the sera of these patients.

Workers at New York University have been investigating the role of Langerhans cells in relation to topical and systemic corticosteroid therapy, and in psoriasis before and after treatment with ultraviolet light. They have noted that the density of Langerhans cells is decreased following topical and systemic administration of glucocorticoids; this decrease is correlated with the concentration and known vasoconstrictive potency of the drugs employed. Patients with psoriasis have normal Langerhans cell density prior to UV therapy; treatment with PUVA or Goekerman therapy decreases Langerhans cell density.

Bullous Diseases

Using a neonatal primary epidermal cell culture model, workers at the University of Michigan are investigating pemphigus and pemphigoid by developing techniques for identifying, isolating and characterizing large quantities of antigens involved in these two bullous diseases. They are also investigating the effects of pemphigoid and pemphigus antibodies on epidermal cell adhesion in tissue culture of epidermal cells. Studies into the basic pathogenetic mechanism of bullous pemphigoid at the University of California at San Francisco have disclosed a unique granule alteration of mast cells that may be characteristic of bullous pemphigoid lesions. They have also suggested that the eosinophil may be the effector cell of this tissue injury.

Workers at the University of North Carolina have been investigating structures at the epidermal-dermal junction which play a role in attaching epidermis to dermis. Disruption of this junctional area occurs as primary pathology in many diseases, including epidermolysis bullosa, bullous lupus erythematosus, and pemphigoid. Using monoclonal antibodies directed at antigens within this dermal-epidermal junction area, workers at the University of Rochester have reported an antibody demonstrating specific reactivity with an antigen that may be altered in recessive dystrophic epidermolysis bullosa. That may provide the basis for a new diagnostic test for this disease. Investigators at Johns Hopkins University have demonstrated the ability to induce IgA circulating immune complexes in patients with dermatitis herpetiformis by wheat feeding, thus further substantiating the association between the gluten-sensitive enteropathy of DH and the bullous skin disease.

Connective Tissues

Many workers are investigating normal collagen synthesis and its modification in various diseases. Studies at UCLA on the synthesis of procollagen by fibroblasts indicate that most pro-alpha chains acquire interchain disulfide linkages in the rough endoplasmic reticulum of fibroblasts and are folded into a triple helical conformation immediately after this event. Extracellularly, this procollagen is efficiently converted into collagen in normal tissues. This requires at least two separate proteinases for the removal of amino-terminal and carboxy-terminal extensions. These workers are also studying degradative aspects of collagen metabolism. They have developed a new technique to demonstrate collagenase activity and have demonstrated low levels of activity in control cell cultures compared to increased levels in cell cultures from a patient with recessive epidermolysis bullosa. These studies are being extended to other diseases of aberrant collagen formation, including scleroderma. Workers at Mount Sinai have been studying collagen and fibronectin synthesis in scleroderma. Cultures from several of their scleroderma patients have shown a 10-20- fold increase in the production of total protein and collagen as well as fibronectin and Type III procollagen as compared to unaffected individuals. Most of these increases were noted in the reticular dermis and at the junction of the dermis and subcutaneous tissue. They have been able to demonstrate increased synthesis of collagen by normal fibroblasts after exposing them to the supernates from stimulated scleroderma mononuclear blood cells. Similarly treated cells from normal controls did not produce this effect. Collagen synthesis was depressed when normal fibroblasts were exposed to the supernate from stimulated normal mononuclear cells. This suggests that the inflammation that occurs in scleroderma may be important in pathogenesis and be more than just a reactive phonomenon.

Acne

A model for both the hyperkeratinizing and inflammatory phases of acne has been developed at the University of California at Los Angeles. The use of several known chloracnegens in the skin of hairless mice induces extensive follicular hyperkeratosis and hyperkeratinization of the dermal pilar cysts, resulting in spontaneous rupture into the dermis to produce pimple-like foci of inflammation. Research at the University of Pennsylvania indicates that bacteria are not involved in the early events of acne, and a critical level of sebum is required before bacterial colonization can occur. These findings complement previous findings at the University of Iowa that the use of oral 13-cis retinoic acid induces a dose-dependent inhibition of sebaceous gland function and that the major activity of this drug in the amelioration of acne is mediated through sebaceous gland inhibition.

Vitiligo

Research in vitiligo is being conducted by a consortium of universities supported by a program project grant. These studies have been highly productive to date and have utilized clinics at two institutions which serve more than 1,000 vitiligo patients per year. They have analyzed a wide range of clinical and laboratory manifestations in these patients, and have evaluated the efficacy of various treatments. Among the highlights of these observations are: (1) Ocular involvement has been found in more than one-quarter of patients studied with vitiligo. However, in most patients these abnormalities have not been associated with functional abnormalities. (2) In a small number of patients with vitiligo or vitiligo and concomitant melanoma, discrete lesions of the macula with loss of visual acuity were noted. (3) The prevalence of vitiligo is increased in patients with uveitis of unknown etiology. (4) Some patients with melanomas also have evidence of uveitis. (5) Patients with melanomas who develop vitiligo seem to have prolonged survival. A randomized trial to further evaluate this observation is being undertaken. (6) A sensitive assay has been devised to monitor levels of both 8-methoxypsoralen and trimethylpsoralen in serum; this assay has been used in the investigation of the photopharmacology of these therapeutic agents. (8) PUVA, when administered in excessive amounts, decreases the density of pigment cells in mice. PUVA is probably not a direct stimulant for proliferation of pigment cells. (9) Injury to pigment cells at the border of white patches in patients with vitiligo was demonstrated. (10) Amelanosis in a chicken model of vitiligo appears to be caused by self-destruction of pigment cells in papillae of feathers. The immune system seems to be involved, and some of these experimental birds exhibit a condition that resembles alopecia areata in humans. An accompanying inflammatory uveitis causes blindness in 40 per cent of the birds. (11) Two-thirds of 326 patients with vitiligo experienced psychosocial problems and expressed concern about other medical disorders being possibly associated with vitiligo.

Other investigations into the pathogenesis of vitiligo conducted at New York University have demonstrated that complement fixing antibodies to melanocytes are uncommon in vitiligo. They appear to be limited to a subgroup of patients suffering from both vitiligo and other auto-immune diseases.

Structure and Function of Skin

Investigators at the University of Washington have investigated a stratum corneum basic protein named "filaggrin" that is derived from a precursor found in keratohyalin granules and aggregates keratin filaments. Excessive and abnormal filaggrin has been found in epidermolytic hyperkeratosis. This disease shows abnormal clumps of filaments in the basal and spinous cells, which may be related to the abnormal filaggrin present. Others at the same institution have established a screening program which provides specific biochemical and ultrastructural diagnoses for many patients with rare inherited connective tissue disorders. The screening program has brought to the attention of the investigators new disorders for further study which may lead to further insights into the pathogenesis of selected diseases. These techniques have provided definitive diagnostic technology for inherited connective tissue diseases such as Ehlers-Danlos syndrome Types IV, VI, VII, and an X-linked form of cutis laxa.

Workers at the University of Iowa investigating eccrine sweat gland function have suggested that the dual innervation of the eccrine sweat gland provides a profound control mechanism for regulating the function of both the duct and the secretory portion of the gland. The structure of the microvasculature of the skin has been investigated at Yale University in several different disorders. All telangiectases fall into two patterns: either a dilatation of the horizontal plexus alone or an aneurysmal dilatation of the capillary loops and the horizontal plexus. Their studies of necrobiosis lipoidica (NLD), which had been considered a cutaneous consequence of diabetic microangiopathy, have revealed a loss of elastic tissue fibers in the necrobiotic areas and the close proximity to these disintegrating elastic fibers of macrophages and mast cells along with plasma cells. Their studies indicate that NLD is not a microvascular disease but rather a disease involving sensitized lymphocytes and macrophages leading to elastic fiber destruction and collagen fiber damage.

MULTIPURPOSE ARTHRITIS CENTERS PROGRAM

Overview

NIADDK initiated the Multipurpose Arthritis Centers program in Fiscal Year 1977 with awards totaling $2.5 million to 15 institutions. Awards were made to nine additional centers in 1978 and 1979 and one new center in 1980. In Fiscal Year 1980, the Institute expended $6.9 million in support of the 25 centers active during the year. The number of centers active in FY 1981 was reduced to 21 with an expenditure of $7.0 million.

Many of the projects under way in these centers could not have been started without the interaction of individuals brought together under the aegis of a Multipurpose Arthritis Center. Examples of major efforts of the centers include research into the genetic bases of arthritis; epidemiologic studies; new advances in health services research (such as cost-effectiveness studies and analysis of disability); new curricula for the education of allied health professionals; and research into new methods (some using computers) of delivering arthritis health care to those least able to obtain it; i.e., the home-bound individual and the resident of the inner city. In addition, many of the centers have programs dealing with research on arthritis in children.

Many centers under the revised NIADDK Application Guidelines include common-use core facilities to improve the efficiency and effectiveness of their research operations. These "special core units" range from programs dealing with basic biomedical research to facilities designed to aid in the evaluation of ongoing programs. Arthritis center grants support over 40 developmental and feasibility studies in the areas of biomedical research, and other programs.

Research

Center grant support is intended to complement ongoing research grant support in a given institution and to stimulate the development of new research projects. Each center possesses a substantial basic and clinical research base, supported by other mechanisms, investigating the causes of rheumatic diseases and their treatment.

Research projects supported by center grant funds are, for the most part, developmental and feasibility studies. These studies are designed to encourage investigators to explore interdisciplinary and highly innovative scientific approaches which may later form the basis of applications for investigator-initiated research grant awards from the National Institutes of Health or other agencies. The activities described in this section, as well as in the rest of this report, are those funded with center grant support.

In a novel investigation at the Arthritis Center at the University of Alabama in Birmingham, attempts are being made to implicate an infectious agent in the etiology of arthritis. Most previous studies of this nature have been largely retrospective; that is, in patients with a definite diagnosis of rheumatoid arthritis after months or years of illness. Potential initiating microbes were no longer cultivatable. Persistent antigens may have been eliminated. This study is restricted to patients with inflammatory arthritis of less than three months duration. Samples are obtained from the nose, genital areas, and synovial (joint) fluids, and cultured to detect bacteria, mycoplasmas, and several types of viruses. While synovial fluids have been free of cultivable organisms, various strains of mycoplasma have been isolated from other tissues in several patients. The center has recently developed a technique using a new biological stain which detects, with high sensitivity, DNA present in living organisms. Future use of this stain for the examination of synovial fluid smears should indicate whether or not organisms may be present, although noncultivable.

In another study at the Alabama center, investigators are trying to develop immunological assays that can detect breakdown products of proteoglycan (a structural component of cartilage) in serum or urine of patients afflicted with cartilage degeneration in osteoarthritis or rheumatoid arthritis. The ultimate goal of this project is to use such assays to predict or monitor exacerbation or amelioration of clinical symptoms. Both rabbit and mouse antibodies against several breakdown products have been produced, and are currently being characterized to determine their specificity. Once these substances are available, parameters for immunoassays will be established using normal human serum to which human articular cartilage degradation products have been added.

Another recently initiated project, at the Boston University Arthritis Center, concerns gonococcal arthritis. The purpose of this study is to develop a model of gonococcal infection in rabbits to determine if the clinical manifestations of disease are linked to specific strains of gonococci. Investigators at the center have established that rabbits can be induced to form arthritis after the injection of gonococci into knee joints. It is interesting that as briefly as two hours after injection, live bacteria could no longer be recovered from joint fluid, in contrast to other types of bacteria, which can be recovered up to 14 days after injection.

At the University of Colorado Health Sciences Center in Denver, studies are in progress on mechanisms of abnormal calcification, that complicates scleroderma and other connective tissue diseases. Center investigators have shown that bone powder implanted in a model system in the rat will develop first into cartilage, then calcified cartilage, and finally into bone within 10 to 14 days. From the 14-day implants, a substance has been discovered which has the property to bind calcium. Current work centers on defining optimal conditions for isolating the calcium-binding substance, determining the kinetics of calcium binding at various stages of the implant, and characterizing the nature of the substances to which the calcium binds.

An investigation in progress at the Michigan center involves a new immunosuppressive drug, which has been shown to inhibit both cellular and humoral immune responses in animals. Since research has demonstrated that this drug is effective in treating the systemic lupus erythematosus-like condition found in New Zealand Black mice, the center developed a protocol for studying the potential therapeutic effect of the drug on SLE patients. Of 10 patients with active SLE who have received the drug, to date, most have shown clinical improvement, and in some, this improvement has persisted even after the drug was discontinued.

In another study of clinical interest, the Dartmouth Multipurpose Arthritis Center is performing an investigation of the effects of daily low doses of prednisone on rheumatoid arthritis. It is hypothesized that a small amount of drug will prevent the progress of the disease without causing the serious side effects that often accompany its use in larger doses. This hypothesis was generated by previous laboratory research in the Dartmouth center demonstrating that low doses of prednisone inhibit the production of collagenase by synovial (joint) cells in tissue cultures.

Researchers at the Dartmouth Multipurpose Arthritis Center are also conducting experiments to develop a new drug treatment for rheumatoid arthritis. They have previously shown that the enzyme, collagenase, can play a role in the formation of joint lesions. They had also found that the drug, retinoic acid, could inhibit the formation of collagenase by synovial (joint) cells in tissue culture. Researchers at the center are now designing *in vivo* animal experiments to test their hypothesis.

A new therapeutic approach, using total irradiation of lymph nodes, for testing patients with severe cases of rheumatoid arthritis unresponsive to other forms of therapy was initiated by a developmental and feasibility study at the Harvard Center. The rationale behind this form of treatment is based on the immunosuppressive effects of radiation. By irradiating only the lymph nodes, the effects of the radiation can be localized to immuno-competent lymphocytes. By eliminating only these cells, it was thought that the nodal irradiation would produce a regression of the arthritis. So far, nine patients have been treated with radiation, and it was shown that while levels of immune cells were reduced in all but two of the patients, the radiation had no effect on the titers of rheumatoid factor or immune complexes. It was also discovered that there was an anti-inflammatory effect shortly after the treatment resulting in fewer swollen joints; this beneficial effect lasted for several months. The temporal association of the suppression of arthritis with the demonstrated diminution of immunocompetent cells suggests a major role for these cells in the pathogenesis of rheumatoid arthritis.

Health Services Research is another feature of center activity. The center at the University of California in San Francisco is examining whether total hip joint replacement operations, in addition to being effective in alleviating pain, are also effective in returning patients to useful employment. The surgical procedure in question, known as total hip arthroplasty (THA) is very costly, involving 75,000 operations a year, at a total price of about one billion dollars. The specific aims of this study are to: define costs; describe the social and medical backgrounds; determine the extent to which THA results in a return to work among working age persons, increased functional capacity among housewives, and increased capacity for self-care; and to look at the association between the medical, functional, and disability outcomes of the procedure. During the first year, 210 patients who had received THAs were admitted into the study. From preliminary analyses of the data, the proportion of patients with work disability three years post-operatively was shown to be nearly the same as the portion disabled prior to surgery.

Many of the Multipurpose Arthritis Centers also conduct programs of epidemiologic research, more fully described in the Epidemiology/Data Systems portion of this Annual Report. Examples include: studies of the genetics of familial rheumatoid arthritis at the University of Alabama in Birmingham; a prospective study of the outcome of rheumatoid arthritis at the Stanford University Center; studies concerned with oral contraceptive use and the development of rheumatoid arthritis, and a population screening study for systemic lupus erythematosus in the Boston center; and an investigation of genetic factors in rheumatic diseases by the Johns Hopkins University Center in Baltimore.

Education

Another integral aspect of the Multipurpose Arthritis Centers program involves the development and evaluation of educational activities designed to facilitate and increase the education of various types of health professionals. The center at Washington University has redesigned and evaluated its entire rheumatic disease curriculum for physical therapists. The center's original two-hour course offered to physical therapy students two years ago has been expanded to a 16-hour curriculum composed of lectures, video-tape demonstrations, and a laboratory in which the students examine, diagnose, and recommend appropriate therapies for patients with rheumatic diseases.

The Arthritis Center at Case Western University in Cleveland has created an instructional resource library devoted specifically to the rheumatic diseases. Library materials deal with a wide range of subjects in formats not available in the medical school library: videotapes, sound/slide presentations on 35 mm slides, and audio cassettes. In addition, the library staff has produced a large number of other materials. All items are available to other centers on loan. They are beginning to develop a comprehensive rheumatology x-ray teaching file. They are considering granting Category I continuing medical education credits for the materials it loans out.

The center at the University of Colorado in Denver, has had initiated a training course in rheumatic disease management for Family Practice Residents. Forty-three residents have received training so far, in week-long courses, consisting of lectures, clinics, ward rounds, laboratory workshops, and research seminars. As part of the evaluation of the effectiveness of this course, with the assistance of the Office of Education Services at the University, students are given a pre-test when they enroll for the course and post-test following its completion. In addition, follow-up visits are conducted at the Resident's hospital to obtain data on how the program impacts on the resident's overall training and work-up and treatment of arthritis patients.

A project to develop an arthritis patient education curriculum is underway at the University of Alabama's Center in Birmingham, using a Delphi technique. Notably, this project involves the cooperation of the Arthritis Centers nationwide. The Delphi method is a survey technique for obtaining a consensus in areas where empirical investigation does not generally yield exact conclusions. The method for this study consists of mailing a sequential set of questionnaires to a group of people identified as having special knowledge of patient education.

The center at Birmingham is studying ways to improve the rheumatology education of nursing students and to evaluate the impact of the changes that are made. The hypothesis of this study is that a short-term intensive experience in rheumatology for student nurses can effect long-term changes in knowledge and beliefs regarding arthritis. All third-year students are assigned three hours of self-instruction in rheumatologic disorders, and a randomly chosen portion of the class is given an additional 17 hours of clinical instruction in rheumatology patient care during a one-week period. The 17 hours of instruction were scheduled during a one-week period and consisted of planning conferences, inpatient care at a rehabilitation center, outpatient clinics, home visits, and patient education classes. A 56-term objective test covering rheumatology and rheumatology nursing care was used to evaluate gains in student knowledge. A factorial analysis has shown significantly higher gain scores for students receiving clinical instruction (experimental group) as compared to students who received only printed self-instruction (control group).

Community Demonstrations

Few studies have evaluated the efficacy of "team care" in the treatment of arthritis. At the Stanford Arthritis Center located in Palo Alto, one such study aims to identify the most valuable intervention variable resulting from team care of elderly arthritis patients in a Veterans Administration Medical Center. The study will test the hypothesis that team intervention will result in improved patient outcomes. Two types of team interaction will be studied: attention delivered by the team and actual services delivered by the team. Cost factors will be evaluated.

An investigation was recently initiated by the arthritis center at the Downstate Medical Center in New York to examine the relationship between chronically ill people of diverse ethnic backgrounds and the health care delivery system. Individuals with either rheumatoid arthritis or osteoarthritis will be studied. The study will focus on the extent to which the cultural background of the individual, in interaction with the culture of the health care system, determines that individual's health behavior. The central assumption of the study is that utilization of the health care system is influenced by conflicts between institutional values of the health care system and the cultural values of the patient's community. It has been found that members of minority groups (in this case, American and West Indian Blacks and Latinos) tend to underutilize the available range of medical resources. The study will examine personal health beliefs and the manner in which they are integrated into their community. The study will also ascertain features of the health care system that the population tends to utilize, especially revisit patterns, which are important in the care of chronic illness. It will also study communications between physicians and minority patients.

Special Core Units

These common-use facilities are designed to increase the effectiveness and efficiency of each center's activities. For biomedical research, there are core units for tissue culture, connective tissue metabolism, immunology, histocompatibility testing, and research involving hybridomas. Core units in other areas are concerned with biostatistics, evaluation methodologies, educational activities and epidemiology.

As an example, the Hybridoma Core Facility at the University of Alabama's Arthritis Center in Birmingham is intended to provide the technical expertise and culture equipment necessary for the production of monoclonal antibodies to antigens of interest. This core facility employs the services of two full-time staff members and makes use of appropriate technical assistance. During its first year of operation, the Hybridoma Core Facility has participated in projects dealing with the detection of human histocompatibility antigens and studies of the characterization of human serum proteins and mouse transplantation antigens. Future plans involve participation in the project, described above in the research component activities, to detect mycoplasma infections in humans as a causative agent in rheumatoid arthritis.

At the center located at the University of Michigan in Ann Arbor, the Tissue Culture Core Unit is aiding projects dealing with connective tissue degradation during chronic disease and the isolation of small proteins from connective tissue cells. The evaluation core unit at this center employs a staff which includes an educational psychologist, a psychometrician, a computer programmer, and a secretary. This core unit has been active in providing assistance for projects dealing with curriculum development, questionnaire design, and data analysis.

The center located at Boston University has a unique core unit devoted entirely to the study of amyloid, a type of protein which can accumulate in body tissues in amounts sufficient to impair normal function and cause varied symptoms including arthritis, heart failure, and gastrointestinal dysfunction. The core unit supports essential personnel, equipment, and animal resources and coordinates a variety of amyloid research studies. These studies include biophysical, immunologic, electron microscopic, clinical and therapeutic projects. As an example, patients are involved in a study to evaluate the therapeutic efficacy of colchicine in systemic amyloidosis. A total of 96 patients have so far been included. Preliminary results show that the one-year and 18-month survival rates with this disease were 76 and 60 percent respectively for those patients receiving colchicine compared to 29 and 19 percent when no specific therapy was offered.

At the Johns Hopkins University Center in Baltimore, a Statistical Core Unit has been established during the past year. This core unit will be involved in all aspects of the center's activities. An example of a current effort is preparing a data base for systemic lupus erythematosus for entry onto a computer. This involves abstracting old patient records and laboratory data as well as entering new patients' visits. Future plans include broadening the data base to include other diseases, such as scleroderma and polymyositis.

Another type of core unit, to provide support for the educational activities of the center, is located at the Dartmouth Center in Hanover, New Hampshire. This core is set up to consolidate and coordinate all instructional materials and media to all center staff members by acting as a clearinghouse for production, accumulation, and distribution. The first portion of this core unit, the computer module, is already in place. The computer is currently being used to store and analyze data and prepare mailing lists and has the capability to store and retrieve information for a patient registry. The next portion of this core unit to be developed will provide self-instruction regarding the choice, development, and use of media within the center and serve as a depository for instructional materials in rheumatology.

SPECIAL PROGRAMS IN ARTHRITIS AND RELATED DISEASES

The following important activities are being developed to address certain specific features of the National Arthritis Acts of 1974 and 1966, and several recommendations of the Arthritis Plan submitted to the Congress in April 1976 by the National Commission on Arthritis and Related Musculoskeletal Diseases

EDUCATION PROGRAMS

The primary goal of the arthritis Education Programs Office is the maximum use of the tools of communication to inform and educate arthritis health professionals about arthritis. Significant progress is being made towards stimulating and assisting development of public, patient and professional education as essential elements in achieving optimal treatment and management of arthritis.

Arthritis Information Clearinghouse Office

A major component of the Education Programs Office is the Arthritis Information Clearinghouse. Established by the NIADDK following authorization by the National Arthritis Act and recommendation by the National Commission on Arthritis and Related Musculoskeletal Diseases, the Clearinghouse has completed its third year of operation aimed at improving the lines of communication among health professionals serving the arthritis patient. The major function of the Clearinghouse is to collect, screen, store, and disseminate information about educational materials and programs on the rheumatic diseases. In addition to this service to providers, the Clearinghouse seeks to acquire a better understanding of information needs, especially the unmet ones of arthritis patients. It communicates these needs to the field and provides support and technical assistance to a network of information providers. In serving as a "broker" to foster the flow of arthritis information, thus helping users to locate and select educational materials, the Clearinghouse refers clients to the appropriate developer or source rather than acting as a distributor of the materials itself.

Since its founding three years ago, the Clearinghouse has grown appreciably by introducing new materials and by expanding the more traditional communication lines. The Clearinghouse database now consists of almost 2,000 records, retrievable by keyword, author, source, publication year, and text words in titles and abstracts. Included in the database are print and audiovisual materials, journal articles, reports and textbooks, among other items. Inasmuch as the database is in place and the mechanism for acquiring and processing materials are established, no further development in this area is required at this time. A cumulative catalogue of 1981 accessions has been produced but was not published during this past fiscal year because of the Government-wide OMB moratorium on publications. Printed and audiovisual materials are being added to the database as they appear; since retrospective searching has been completed, the rate of acquisitions in these areas has declined. An increased effort to identify and describe educational programs and incorporate them into the database continues to be a major priority for the coming fiscal year.

Products and Services

Clearinghouse products thus far include about 35 bibliographies and catalogues, a Thesaurus, and a cumulative catalogue of accessions, while its services, approximately 400 per month, include requests for publications, mail list additions, database sources and referrals. DHHS approval was obtained (one of the very few in the Department during this past fiscal year) for a catalogue on Patient Education Resources. This catalogue is intended to provide health professionals with an updated and expanded guide to printed patient education resources on rheumatic diseases.

Due to a heavy demand of requests for literature searches and in order to streamline requests, packaged bibliographies on frequently requested topics have been prepared. Several directories ahve been assembled to speed answers to information and referral questions. Approximately 18,000 Clearinghouse products have been distributed during the past fiscal year.

Cooperation with National Arthritis Advisory Board

The Education Programs Officer and the Clearinghouse staff have evolved a close working relationship with the Subcommittee on Education and Training of the National Arthritis Advisory Board. Two special survey reports, The Training of Medical Students in Interpersonal Relations and The Training of Medical Students in Pharmacology, have been prepared for NAAB.

Outreach

The Clearinghouse has continued its active outreach program, contacting conferences and meetings and providing exhibit materials and package enclosures. A short slide talk explaining Clearinghouse activities has been presented at some meetings. As part of its outreach program, periodic memoranda are being published to alert users and health professional constituents as to new developments. In addition, a Clearinghouse exhibit has been sent to meetings and conferences, including the annual meeting of the American Rheumatism Association, the Medical Library Association, and regional arthritis-related meetings.

Cooperation with Multipurpose Arthritis Centers

The Clearinghouse has continued its efforts to collect information on community and institutional patient education and in-service training programs within the Multipurpose Arthritis Centers. The Clearinghouse continues to act as a depository for MAC reports, reprints, and studies. Curricula and program descriptions of patient education or in-service training classes are being collected, and materials developed such as syllabi, resource lists and supplementary materials are being sought.

Advisory Group

An advisory group comprised of eleven rheumatologists, educators, library scientists and other health professionals has continued to help the Clearinghouse in effective planning and accomplishment of goals. The group, meeting twice yearly, provides recommendations to the Clearinghouse project officer and staff.

Transition

During this fiscal year, a new contract support group replaced the original group. Herner and Company arranged for the inventory and removal of the Clearinghouse materials from Capital Systems Group to the AIC's new headquarters at Herner and

Company in Arlington, Virginia. The project officer and Herner personnel visited Capital Systems Group offices to inspect the physical arrangement of the AIC material and arrange for its orderly transition to the Herner site.

COOPERATIVE SYSTEMATIC STUDIES OF RHEUMATIC DISEASES

The Division also supports cooperative multi-centered studies on various aspects of rheumatic diseases, usually on projects that require the resources of more than a single institution. Major emphasis is given to carefully designed clinical trials of potentially therapeutic agents. These studies are coordinated by central facilities and research personnel at the University of Utah, and conducted at eight participating research centers, which were selected competitively. An advisory committee meets regularly to choose the most promising study proposals, and to review, improve and approve the design of study protocols before an individual trial is initiated.

The Cooperative Clinics recently completed a study comparing auranofin(a new oral gold salt preparation), with parenteral gold salt treatment (an "established" remitting agent) and placebo in the treatment of 163 patients with rheumatoid arthritis. Both types of treatment with gold, as compared to placebo, demonstrated significant improvement with respect to the number of tender joints, joint tenderness score, and physician assessment. Patients treated with parenteral gold sodium thiomaleate (GTM), had improvement in duration of morning stiffness and joint swelling score, compared with placebo, while auranofin-treated patients did not show this improvement. There was no significant difference between the three treatment groups with respect to grip strength, number of swollen joints, patient assessment of disease activity, and functional class. Several patients, particularly those treated with GTM, had adverse reactions and were withdrawn from the trial. Thus, in the doses used, auranofin may prove to be a useful therapeutic agent for rheumatoid arthritis.

Another recently completed trial was the evaluation of low dose pulse methotrexate (MTX) in the treatment of patients with severe, unresponsive psoriatic arthritis. Patients were maintained on prior agents, such as ibuprofen or indomethocin. They were randomly assigned to pulse-treatments of MTX or to placebo. At the end of 12 weeks, the group taking MTX had significant improvement in terms of physician's assessment and the area of skin involvement. Joint tenderness and swelling improved with MTX, but the differences were not statistically significant. Neither the MTX group nor the placebo group had serious side effects. The data suggest that MTX is well tolerated in the low dose, pulsed method and is of some benefit in treating psoriatic arthritis.

The Cooperative Clinics are also now evaluating the efficacy and risks of dimethylsulfoxide (DMSO) in digital ulcers of scleroderma, and of pulsed-intravenous methylprednisolone therapy for lupus nephritis.

ARTHRITIS EPIDEMIOLOGY PROGRAM

The Epidemiology Program Office provides an administrative focus for efforts to encourage epidemiologic research in the field of arthritis and related musculoskeletal diseases. The office also facilitates cooperation and exchange of statistical information among investigators. During the past year, the number of research grant applications with primary emphasis on arthritis epidemiology has increased. Resources for epidemiologic research within the Multicenter Arthritis Centers have expanded. In addition, increased attention to undertaking methodologically sound population-based studies has occurred within the ARAMIS consortium.

Epidemiologic Activities in the Multipurpose Arthritis Centers

Six centers have statistical and epidemiologic core units designed to enhance efficiency and effectiveness of the center's activities. The Stanford Biostatistical, Computer, and Education Methodology Core Unit, for example, provides consultation and review of all projects. It offers to center personnel expertise in biostatistics, computational consultation, intervention methodology, outcome measurement, and independent assessment.

At Brigham and Women's Hospital MAC, the Core Unit for Clinical Epidemiology and Evaluation in the Rheumatic Diseases, assists in formalization of research questions, aids in design of studies, and provides computerized methods for data storage and retrieval; and assists in statistical analyses.

The Research Evaluation and Support Core Unit (RESCU) at Boston University focuses on development and application of the Arthritis Impact Measurement Scales instrument. RESCU also works to coordinate and organize analyses of research data within the MAC. Data on amyloidosis, an area of special research emphasis in this center, are being computerized within this core.

The centers also help development of individuals skilled in pursuing epidemiologic studies of rheumatic diseases. At the Johns Hopkins Center, a postdoctoral fellowship is available for a three-year program leading to a Masters of Public Health degree in epidemiology and full training in rheumatology. Epidemiologic training programs are also offered at the Rosalind Russell MAC (San Francisco) and at the Brigham and Women's Hospital MAC (Boston). In addition, epidemiologists familiar with other chronic diseases are being recruited. These programs may help to nurture the interest of young investigators in careers of rheumatic diseases epidemiology.

Yersinia and Reactive Arthritis

Epidemiologic studies of the occurrence of reactive arthritis consequent to *Yersinia* infection are being carried out by Emory University investigators at the Yerkes Primate Center, as part of a larger investigation assessing the relationship between *Yersinia* infections and subsequent development of arthritis. Yersiniosis is endemic in the Yerkes Field Station colony of nonhuman primates, where there is an increasing frequency of acute arthritis. Investigators will systematically screen the animals housed in selected enclosures. In addition, the entire population of Yerkes colony will have bacterial cultures to test for *Yersinia* and other enteric pathogens. Serial stool cultures and other clinical studies will be carried out on ill animals and synovial biopsies will be performed to determine the presence of organisms or antigens. The frequency of *Yersinia* in trapped wild rodents at field stations in different areas of the colony will also be carried out. Preliminary studies indicate that rats and mice are natural reservoirs for *Yersinia*. Cultured bacteria from rodents and primates will be identified and serotyped. These primate studies are designed to measure the frequency and distribution of *Yersinia* and to identify specific serotypes associated with reactive arthritis.

Lyme Disease

Evidence leading to the discovery of the etiologic agent of Lyme Arthritis was collected by researchers at Yale. Lyme Arthritis was initially recognized in 1975 as a separate entity because of geographic clustering of children with intermittent, oligoarticular arthritis in Lyme, Connecticut. Yale investigators identified a unique clinical marker, a skin lesion (erythema chronicum migrans (ECM), which preceded other manifestations of illness. Analysis of patients with Lyme Arthritis suggested transmission of a causitive agent by an arthroped vector. In 1977 a newly-described tick species, *Ixodes dammini*, was identified as that vector. Studies suggested that antibiotic therapy shortened the course of ECM and prevented or attenuated subsequent arthritis. Improvement of symptoms following penicillin therapy aided in directing attention toward a penicillin-susceptible bacterium as a causative agent. A spirochete causative agent was recently reported by National Institute of Allergy and Infectious Disease's (NIAID) Rocky Mountain Laboratories. The significance of the spirochete in Lyme Arthritis and its relationship to the *Ixodes dammini* tick can now be addressed through further epidemiological studies.

Osteoporosis and Fractures

In a Rochester, MN population, the overall risk of recurrence of hip fractures was 29% by 20 years after the initial fracture. This figure was nearly three times the expected rate. In another study of the same population, Colles' fractures, which are associated with minor trauma, were found to be predictive of an overall 50% increase in the risk of subsequent hip fracture.

These recent findings are from an epidemiologic study of postmenopausal women in a circumscribed community. They are part of a continuing Mayo Foundation effort to characterize women who are at risk for excessive bone loss and for spine and hip fractures. This information could identify individuals in need of prophylactic therapy and aid the design of preventive programs to reduce the occurrence of age-related fractures.

Epidemiology of Scoliosis Workshop

Scoliosis is a lateral curvature of a segment of the spine lacking normal flexibility. It is most frequently seen in otherwise healthy children and usually manifests itself during early adolescent years. Progression, which is extremely variable, may lead to unequal shoulder height, a deformed waist, torso tilt, and unilateral rib hump in the back. While the etiology of most types of scoliosis remains unknown, familial studies have suggested that genetic transmission plays a significant role.

Untreated severe scoliosis is associated with an increased mortality rate, a high frequency of back pain, a greater risk of osteoarthritis, and negative psychosocial sequelae. Prompt treatment of children with small progressing curves is reported to prevent significant deformity in more than 85 percent of cases.

Screening programs could provide an outstanding resource for epidemiologic studies by exploring the natural history of the disorder, identifying some inherent risk factors, and aiding in the development of a means of identifying which spinal curves are most likely to progress.

A multi-disciplinary group of orthopedists and epidemiologists, plus individuals with expertise in bioengineering, screening and specific data resources attended a mid-July 1982 Epidemiology of Scoliosis Workshop. The objective was to generate suggestions for further studies and develop testable preventive strategies, with the ultimate aim of minimizing disability.

The workshop focussed on four major topics: 1) the natural history and underlying etiologic hypotheses, 2) the state of epidemiologic knowledge, 3) epidemiologic methods and approaches which might be most appropriate, and 4) existing and potential data resources to carry out epidemiologic studies.

ARTHRITIS DATA SYSTEMS PROGRAM

The Data Systems Program of NIADDK/AMSD fosters systemic acquisition, storage, retrieval, and analysis of information concerning rheumatic diseases. Major effort is concentrated on assuring validity, confidentiality and comparability of data collected in separate institutions, and integrating data resources with data needs. During the past year, developments related to rheumatologic data collection and analysis have been prominent in three major settings: 1) American Rheumatism Medical Information System (ARAMIS) consortium administered from Stanford University, 2) Health and Nutrition Examination Survey (HANES I) Epidemiologic Follow-Up coordinated by the National Center for Health Statistics, in collaboration with NIH, and 3) Multipurpose Arthritis Centers.

A National Arthritis Data Source

ARAMIS is a multi-institutional databank system for rheumatic diseases. It is an interactive computer system that presently consists of 17 separate databanks which contain clinical information on more than 90,000 patient visits on 17,000 patients. Databanks size has increased by approximately 20 percent over the past year. Three new databanks have been added.

ARAMIS represents the largest body of information on patients with rheumatic disease ever accumulated. The technical capability to manipulate and retrieve these data is provided by an advanced data management system (TOD). Access is available over a communication network throughout North America. Over 40 investigators and over 60 specific projects are involved in ARAMIS.

Goals of ARAMIS include: 1) improved diagnostic classification of rheumatic diseases, including establishment of acceptable criteria; 2) identification of "risk" factors associated with good or poor outcomes based on prognostic studies among defined patient subsets; and 3) improvement of evaluation instruments for these purposes.

Increased attention has been given to studies of prognosis. For example, Health Assessment Questionnaire (HAQ) developed and validated at Stanford, is presently being used in studies of long-term impact of disease on patients with rheumatoid arthritis in term of mortality, disability and cost. Also at Stanford, a system has been developed to follow outcome for patients who have undergone total joint replacement. It comprises a computerized record system which incorporates the in-patient hospital record with a long-term outcome questionnaire. A patient self-evaluation assessment instrument is being prepared for those unable to return for post-operative evaluation. ARAMIS studies include: the effect of chlorambucil in SLE nephritis; the effect of methotrexate in rheumatoid arthritis; the effect of anti-hypertensive drugs in treatment of scleroderma; the effect of d-penicillamine in treatment of scleroderma, and the potential disease modifying effects of certain non-steroidal, anti-inflammatory agents. ARAMIS has continued to play an important role in evaluation of disease criteria. Recently completed classification studies for Juvenile Rheumatoid Arthritis Criteria and Revised 1982 SLE Criteria will be published in the near future. Classification studies of psoriatic arthritis, seronegative rheumatoid arthritis, vasculitis and myositis myositis are in progress. The fourth annual ARAMIS Databank Seminar provided an opportunity for approximately 100 participants (including representatives from seven of the Multipurpose Arthritis Centers) to examine many critical questions related to rheumatologic data acquisition and analysis. Among the subjects addressed in depth were: outcome measurement and the compression of morbidity; strength and limitations of databank research; data quality; experimental design; defining investigative hypotheses; and statistical concepts. Reports on databank research projects were presented and critiqued.

Health and Nutrition Examination Survey (HANES) Epidemiologic Follow-up Survey

In cooperation with the National Institute on Aging (NIA), the National Center for Health Statistics (NCHS) and several other Institutes, the Arthritis Epidemiology and Data Systems Program has contributed actively in the design of the instrument for collection of data in this resurvey of 14,407 individuals, originally sampled in 1971-1975.

Careful preparation of the arthritis section of the follow-up instrument will serve four principal objectives of the project which are: retrospective morbidity and mortality associated with suspected risk factors; prospective mortality associated with suspected risk factors; changes in patient characteristics between HANES I and current status; and natural life history of chronic disease and functional impairments. As of June 1982, the questionnaire had been successfully field tested, and the survey was in progress.

The Multipurpose Arthritis Centers (MACs)

MACs are increasingly developing data instruments that can promote uniformity in studies of the chronic rheumatic diseases. One example, the Patient Self-Assessment Questionnaire (PSAQ), was designed to identify need for specific allied health professional interventions for arthritis patients. Developed and tested over a two-year period at the University of Michigan MAC, it has now become a routine admission procedure. Another achievement is the development of the Arthritis Impact Measurement Scales (AIMS) at the Boston University Center. This self-administered instrument includes the following dimensions: mobility, physical activity, social activity, social role, activities of daily living, pain, dexterity, anxiety and depression. The questionnaire's reliability and validity have been tested in a number of small populations. Comparison of the AIMS with the Stanford HAQ showed close agreement. Studies of the serial use of either of these instruments has demonstrated their sensitivity in measuring change in patient status.

ANNUAL REPORT FOR THE
DIVISION OF DIABETES, ENDOCRINOLOGY, AND METABOLIC DISEASES

OFFICE OF THE ASSOCIATE DIRECTOR- Ernest W. Johnson, Ph.D. (Acting)

This Office plans and directs programs of research grants and contracts, training and fellowship awards, and special programs and activities within the Diabetes, Endocrinology, and Metabolic Diseases Division. The Division Director is responsible for strengthening, integrating, and coordinating activities within these areas and for developing and initiating new programs to meet the needs of the scientific community.

In the area of diabetes, the Division Director, acting through the NIH Diabetes Coordinating Committee, has primary responsibility for coordination of all trans-NIH activities relating to diabetes mellitus and its complications. This provides a centralized focus for the planning, development, implementation, and assessment of program initiatives among each of the NIH Institutes that support diabetes-related research.

The Division Director also serves as Chairman of the Diabetes Mellitus Interagency Coordinating Committee, whose membership includes representatives from all Federal agencies which conduct or support activities related to diabetes mellitus. He also serves as an ex officio member of the National Diabetes Advisory Board, and plays a lead role in liaison with voluntary health groups and scientific societies concerned with diabetes, endocrinology, and metabolic diseases. Thus, he is frequently and intimately involved with the activities of such groups as the American Diabetes Association, the Juvenile Diabetes Foundation, the Cystic Fibrosis Foundation, and the Endocrine Society.

The Division Director also is actively involved with various special activities related to diabetes, endocrinology, and metabolism. During the past year, particular emphasis has been given to implementing the first phase of a multi-center clinical trial to assess the relationship between blood glucose control and prevention or amelioration of the early vascular complications of insulin-dependent diabetes. In addition, emphasis has been given to further improving relationships among NIADDK, grantee institutions, and the private sector through frequent meetings, on site visits, and informal discussions. Through such interactions, the Division has expanded its efforts to facilitate effective utilization of available resources within both the public and private sectors in order to ensure that the highest priority needs and opportunities for research in diabetes, endocrinology, and metabolism are both identified and pursued.

During the past year, Division staff have cooperated with and provided limited technical assistance to the National Diabetes Research Interchange (NDRI), a privately sponsored organization initiated in 1981 under the auspices of the Juvenile Diabetes Foundation. The NDRI has established facilities for providing investigators with human tissues, both normal and diabetic, to be used for research purposes. To date, several dozen investigators supported by the NIH have received tissues from this organization, including kidney, pancreas, eye, and umbilical cord tissues.

Although not detailed below, Division staff also serve as an information resource to the scientific community, lay public, and other Federal, state, and private organizations with respect to the full spectrum of research, training, and information-transfer activities in diabetes, endocrinology, and metabolism. In addition, they contribute to a wide variety of NIADDK and NIH-sponsored activities to further the continued development of expanded scientific capabilities in other related areas of biomedical research.

DIABETES PROGRAMS BRANCH

This branch administers the Division's Diabetes Research and Clinical Trials Programs. In addition, the office of the Branch Chief provides an administrative focus for planning and implementing special initiatives and for coordinating support of diabetes-related awards in other NIADDK programs.

Diabetes Research Program

During the past year, the Diabetes Research Program supported research grants and contracts in a variety of scientific disciplines which collectively contribute to the base of knowledge related to diabetes mellitus and its complications. Progress continued in various areas which are fundamental to the ultimate development of improved medical capabilities for prevention, diagnosis, treatment, and cure of this condition. Some areas of particular research interest include the following: segmental pancreas and islet cell transplantation; insulin delivery systems; the epidemiology of diabetes and its complications; the biosynthesis, secretion, action, and interaction of insulin and other hormones; the pathophysiology of pancreatic islet cells; abnormalities of carbohydrate, lipid, and protein metabolism associated with diabetes and its complications; the possible relationship between diabetes and various genetic, viral, immunologic, toxicologic, and nutritional parameters; and the psychosocial aspects of diabetes. Recent progress in some of these areas is summarized below.

Transplantation

The ideal method for treating the lack of insulin production seen especially in Type 1, or insulin-dependent diabetes mellitus (IDDM), would involve replacement of the pancreas or its defective islet cells. One of the most critical problems limiting the development of such procedures has been rejection of islet cell or segmental pancreas transplants by the host's immune system. However, as described in last year's Annual Report, several techniques recently have been developed which can prolong the survival of rat islet allografts across major histocompatibility barriers without the need for continued administration of immunosuppressive agents. During the past year, these studies have been extended and several new procedures have been developed which permit the successful transplantation of isolated islet cells. In this regard, a specific antigen (Ia) on a mouse islet cell transplant was identified as being responsible for initiating rejection of the islet cells by the host. Subsequently, an antibody to this antigen was developed which allowed for the successful transplantation of the islet cells. This study demonstrated that some type of suppressor cell activity or tolerance had been induced in the recipients bearing the allografts. This in turn led to studies which involved pretreatment of recipients with another type of Ia negative cell, the mouse red blood cell. Donor blood was incubated with specific Ia antisera and complement, washed, and injected two weeks before islet transplantation. Ten of twelve transplants were not rejected 100 days after transplantation in experimental animals. These findings are of particular importance since they suggest that immunization with Ia negative cells will produce a tolerant status in the recipient so that it is possible to transplant islets that have some degree of contamination with Ia positive cells.

In the past, whole pancreas transplantation has been attempted in humans but without much success. One of the main problems was the management of exocrine secretions. Recent approaches involve the use of immediately vascularized segmental pancreata with injection of synthetic polymers in the duct to ablate the exocrine function. The use of a silicone rubber adhesive which has been approved for human research for other purposes, and extra-peritoneal placement of the pancreas to absorb residual secretions, have resulted in some success in experiments involving human patients who already have a kidney transplant and have already assumed the risk of immunosuppression.

Insulin Delivery Systems

The Program continued to support the development and evaluation of both extracorporeal and implantable automated insulin delivery systems for use in biomedical research. Recent progress in this area has been encouraging and active interest by the private sector has demonstrated that a variety of automated insulin delivery systems are now, or will soon be, marketed. As stated last year, the program's contracts for the development and refinement of insulin delivery systems will soon meet their stated goals. As the majority of these projects move to completion, interest has been expressed by private industry in manufacturing and marketing some of the devices which have been developed with program support.

Now that many of the mechanical and technologic aspects of pump development have been solved, more attention has been focused on the problems of insulin aggregation as well as mode and site of hormone delivery. Several investigators have utilized solvents such as glycerol, or additives such as bicarbonate, in an attempt to eliminate aggregation. Other investigators have turned to sulfated and other substituted insulins as a means of preventing precipitation and clogging problems. In addition, there are ongoing studies on the effect of various modes of delivery (intraperitoneal, intravenous or subcutaneous) on the development of insulin antibodies, maintenance of blood glucose control, alterations in insulin requirements, and the social impact on patients of the various insulin delivery devices. The program will continue to support new studies in these and related areas through the investigator-initiated research grant mechanism.

Hormone Action

Recent studies have shown that insulin at very low concentrations decreases the activity of mRNA that codes for phospho-enolpyruvate carboxykinase (PEPCK) a critical rate-limiting enzyme involved in glyconeogenesis. This effect has been demonstrated both in chow-fed rats made diabetic with streptozotocin and in a cultured hepatoma cell line. The effect in the hepatoma cells can be seen in the absence of other added hormones, but is more rapid if the activity of the mRNA is first increased by reaction with cAMP or a glucocorticoid. Thus a direct link has been demonstrated between insulin receptors and the effect of insulin on a specfic mRNA. These investigators have also initiated studies of the structure of PEPCK gene and are in the process of screening their DNA library for cDNAs that can be used to synthesize a full length copy of the gene.

Insulin Receptors

Resistance to the actions of insulin is a characteristic feature of both obesity and non-insulin-dependent diabetes mellitus (NIDDM). Although the resistance may be caused by a variety of factors, the demonstrable consequence is hyperglycemia, especially in NIDDM patients. Sulfonylureas have been utilized in the treatment of NIDDM because they have been shown to exert a hypoglycemic effect in NIDDM. The mechanism through which oral sulfonylureas exert their extrapancreatic hypoglycemic effect has been studied in recent years. Previous evidence suggested that the sulfonylurea tolazamide potentiates insulin action through the enhancement of insulin-stimulated hexose transport. Recent studies from one group suggest that a second generation sulfonylurea, glyburide, also enhances insulin-stimulated glucose transport and that the main action of this class of drugs is to alter post-receptor rather than receptor events. In experiments where male Sprague-Dawley rats were fed either no drugs or tolazamide for a period of ten days, fasting glucose levels were markedly reduced in the treated group versus the control group. However, plasma insulin levels had not changed significantly, suggesting an extra pancreatic, hypoglycemic effect of the sulfonylurea. When the enzyme transglutaminase (which may play a role in insulin-induced receptor down-regulation) was examined in the post-nuclear fraction of both liver and fat cell homogenates, the activity was the same in both groups of animals. In addition, the binding of insulin to purified liver membranes was unchanged by sulfonylurea treatment. These observations suggest that sulfonylurea induced hypoglycemia is not due to changes in either the insulin levels or in insulin binding and that the drugs alter post-receptor insulin action without influencing the receptor per se.

Another group examined the distribution and mobility of the insulin receptor on the plasma membranes of liver cells and adipocytes. It was found that receptors on liver plasma membranes (LPM) exist as single sites, while those on adipocyte plasma membranes (APM) exist in small groups. LPM and APM were incubated with ferritin labeled insulin, and with and without unlabeled insulin, at various concentrations in order to confirm this distribution. When these same cells were incubated with labeled insulin in the presence of anti-insulin IgG (AIAb), it was shown that AIAb produced a significant redistribution of occupied receptor sites in LPM. Occupied receptors on LPM with AIAb revealed over 60% of receptors as single sites; after treatment with AIAb, only 30% of receptors were found as single sites. In contrast, APM occupied receptors, found to be distributed as small groups, were unaffected by the presence of AIAb. These data show that insulin-occupied receptors are mobile and that insulin itself does not cause receptor aggregation. The difference in receptor distribution in the presence of AIAb suggests that there may be unique differences in the liver and adipocyte cell membranes or their insulin receptors, or both.

Hormone Synthesis

Recent advances in recombinant DNA techniques have allowed for structural studies of the human insulin gene. A gene consists of a combination of coding and non-coding nucleotide base pairs. Each nucleotide has a 5' and 3' end so that when they interlock to form a sequence, the newly formed sequence then has a 5' and 3' end. The 5' end is thought to house the promoter, the end at which mRNA transcription is initiated. Recently a large fraction of human DNA was cloned and 1430 base pairs containing the insulin gene were sequenced. In addition, 5650 base pairs on the 5' side and 11,500 base pairs in the 3' end of the gene were analyzed by restriction endonuclease mapping.

Restriction endonucleases are enzymes which recognize specific base pairs of DNA and reproducibly cut at those base pairs whenever they are encountered. This technique was used to study the arrangement of the human insulin gene in a small group of individuals. Insertions of 1.5 to 3.4 kilobase pairs in the 5' flanking region of these genes were found in the DNA of 43% of these individuals. These insertions occurred within 1.3 kilobase pairs of the transcription initiation site. The prevalence of these insertions was significantly greater in NIDDM diabetics (66%) than in non-diabetic groups (29%). No such insertions were found near the 3' end. The existence of these insertions near the 5' end or promoter region of the gene suggest that these insertions may play a role in insulin gene expression (transcription of mRNA).

Previous studies with various insulin analogs have shown that receptor binding affinities and in vitro biologic potency were similar to that of the natural hormone. Most biologically active analogs exhibited the same maximal activity and the same receptor binding ability as insulin. New analogs have been developed which show that present ideas regarding biological activity and receptor binding may need re-evaluation. One analog, which substitutes A^2 isoleucine for norleucine caused a dramatic and unanticipated loss of receptor binding affinity and a decrease in biologic activity. The data suggest that although the A^2 isoleucine analog of insulin has a reduced affinity for the insulin receptor, once bound, the analog-receptor complex is fully capable of initiating events which lead to biological activity.

Another analog, in which the C-terminal amino acid of the A-chain asparagine is replaced by asparaginamides, has the same maximal activity as does native insulin. However, in receptor binding assays, this analog demonstrates a four-fold increase in biologic potency. Several models could explain this phenomenon and additional research will be necessary to determine the possible correlations between chemical structure, conformation, biological activity, and binding affinity of such analogs. Clarification of the effect of these changes will help elucidate the mechanism of insulin action and could lead to the synthesis of analogs which could possess more specific biological properties than the natural hormone.

Hormone Secretion

Obesity is characterized by hyperinsulinemia in both rodents and humans. The increased level of insulin is a result of the rate of insulin secretion and changes in its metabolic clearance rate. The pituitary gland secretes a variety of hormones that regulate various endocrine secretions and a peptide from this gland has been sought which affects insulin secretion. A technique recently has been perfected in which the isolated rat pancreas is perfused directly with the secretions from the pituitary of ob/ob mice (animals that are characteristically hyperinsulinemic), and insulin release and insulin response is measured. The secretions from the intermediary lobe of the pituitary yielded a purified fraction which stimulated insulin release and reacted immunologically with a part of corticotropin, known as CLIP. CLIP does not have B-cell tropic activity, but the ACTH, which is secreted by the adrenal gland, is used in the treatment of ketosis in animals. The purified fraction from the pituitary also stimulated fat synthesis in cultured adipocytes. A similiar factor has now been identified in the plasma of the ob/ob mice and in some obese humans, but not in lean human controls.

Secretion of insulin from the B-cell is also thought to be affected by inhibitory influences such as elevated levels of circulating insulin. A study was recently conducted to look at such an effect by using a hyperinsulinemic, euglycemic "clamp" technique with secretion being measured by assessing C-peptide levels. This study demonstrated that during stable arterial euglycemia, physiological increases of insulin suppression occurred in both lean and obese subjects; however, the C-peptide levels remained several times higher in the obese subjects than the lean. This condition could be due to an increased B-cell mass of normally secreting cells, to a normal B-cell mass of hypersecreting cells or to a combination of the two situations. Future studies are planned to confirm which is correct but the indications are that hyperinsulinemia seen in obese subjects may be mediated in some way by the inability of insulin to suppress its own secretion to the same level as that seen in lean subjects.

It is well known that three pancreatic hormones, insulin, glucagon, and somatostatin function synergistically and/or antagonistically in the regulation of plasma blood sugar levels. Receptors for each of these hormones are thought to be present on the various types of cells found within pancreatic islets. Recently one group has attempted to visualize these receptors on islet cells by quantitative electron microscopic autoradiography. Utilizing radiolabeled insulin, glucagon, and somatostatin, attempts were made to characterize the type of radioligand found on the insulin (B), glucagon (A) and somatostatin (D) cells of isolated islets. Insulin-related grains were more prominent on B cells (60%) than on either A cells (35%) or D cells (27%). Glucagon-associated grains were found most frequently on B cells (40%) compared to 31% on D cells and 15% for A cells. Labeling with somatostatin produced grains in A cells (73%), B cells (37%) and D cells (33%). These studies suggest that the receptors for each of the three islet cell hormones are present on each of the islet cells corresponding to the hormone. The highest density labeling of B, D and A cells by the radioligand, however, correlates well with the known actions of the three hormones on each of the islet cells. This technique provides a powerful tool for investigating hormone receptors on individual islet cells.

Diet, Exercise and Nutrition

One of the main functions of insulin is to maintain carbohydrate homeostasis. The lack of insulin or a defect in its action is associated with either IDDM (Type I) or NIDDM (Type II). Type I diabetes is treated with insulin while Type II diabetes can be treated with diet, exercise, and insulin or other drugs. Exercise appears to make the body more responsive to the available insulin. Recent studies have shown that exercise increases the insulin sensitivity of the fat cells. These studies were performed on an exercise trained (ET) group of rats and on a sedentary (S) group. Fat cells from the ET rats were smaller than those of the S rats and the cells of the ET rats bound more insulin and had higher rates of glucose uptake and oxidation. The increased sensitivity and responsiveness to insulin did not appear to be a result of increased insulin binding solely, but appears to affect a step beyond binding.

Another study demonstrated a relationship between diet and insulin sensitivity in the isolated soleus muscle of 10-week-old lean Zucker rats. Rats were fed for 10 days with a high fat or a high carbohydrate diet that had 67% calories as fat or carboydrate respectively. Plasma insulin, but not plasma glucose concentrations, were significantly elevated in high-fat fed rats indicating a state of insulin resistance. Insulin binding, glucose uptake, and glucose metabolism were measured. High-fat fed rats showed a decrease in insulin receptor number. Glucose uptake was also decreased in these animals and specific alterations in basal and insulin-stimulated glucose metabolism were observed.

Complications

Diffuse impairment of neural function (polyneuropathy) is one of several types of diabetic neuropathies. A recent study investigated one of the metabolic theories used to explain the relationship between plasma glucose concentrations and diabetic nerve disease. Motor and sensory nerve conduction studies were performed on patients with NIDDM whose plasma glucose levels were steady but elevated and who were not under treatment. None of the patients had systemic neuropathy. In these patients, all motor and sensory nerves tested had abnormal rates of conduction, with the greatest abnormality in the motor nerves. All of the motor nerve abnormalities were directly correlated with fasting plasma glucose concentrations and glycosylated hemoglobin levels. Sensory nerve abnormalities were not correlated with fasting plasma glucose or glycosylated hemoglobin despite the fact that sensory and motor nerve conduction velocities correlated with one another. This is the first study to show a relationship between the degree of glucose response to treatment and degree of change in nerve performance. While sensory nerve impairment did not correlate with plasma glucose, the evidence suggests that myoinositol is involved in this abnormality. As reported last year, patients treated with myoinositol had improved conduction in sensory nerves but not motor nerves. Resolving the differences in these two manifestations of polyneuropathy will be important in devising treatments for them.

Immunology and Genetics

Many studies have shown cell mediated immunity in the pathogenesis of B cell loss in the development of IDDM. The spontaneously diabetic BB Wistar rat has proven to be a useful model to investigate this phenomenon. The presence of islet cell surface antibodies (ICSA), thought to play a role in the pathogenesis of human B cells, has been demonstrated in Wistar rat islet cells. ICSA and lymphocyte antibodies (LA) were found by one group in 14 diabetic rats 3 to 11 days after onset of the disease (ICSA, N=12 and LA, N=13). Rat litters with a high incidence of diabetes produced 21 animals who were lymphopenic and showed insulitis, and 20 of the 21 had ICSA. Seven in this group developed IDDM, five of whom demonstrated ICSA before onset while another six animals who developed glucose intolerance (IGT), either showed ICSA at the time of IGT or soon after. Studies in litters with low incidence of ICSA did not identify any animals with IDDM, and only transient ICSA, usually associated with IGT or insulitis. These studies support the conclusions that insulitis is associated with ICSA, and that IGT may exist before the development of ICSA in rats. The presence of ICSA and LA in diabetic rats supports a possible pathogenic role of the antibodies in the development of the disease.

A group working with the BB Wistar rat has attempted to identify the roles that rejection and autoimmunity may play in the destruction of islets following their transplantation into a diabetic animal. When islets from normal Wistar-Furth (WF) rats were transplanted into spontaneously diabetic BB Wistar rats, they were destroyed within a few days; however, it was not clear whether destruction was the result of rejection or an autoimmune process. Immunosuppression caused extended survival of islet transplants, but the increased survival could have been due to interference with either process.

When BB rats were made tolerant to WF tissue thus inhibiting the rejection process, the survival of transplanted islets was not materially improved; however, if the rats were made generally immunologically tolerant through the use of bone marrow transplants before the onset of the disease, the incidence of diabetes was significantly reduced. These results suggested that the primary process of islet destruction in BB rats after transplantation was through an autoimmune mechanism rather than rejection and also that methods may one day be devised to prevent diabetes in humans by immunologic manipulations in individuals identified as being diabetes-prone.

Epidemiology

Large scale epidemiologic studies allow for the examination of a multitude of factors relating to diabetes such as genetic markers, susceptibility to developing diabetes and complications, role of environmental factors, obesity, exercise, diet, and socioeconomic status. They also allow for the prospective examination of the relationship of these factors to the development of diabetes.

Recent work by one group has shown increased risk for developing NIDDM associated with HLA-BW61, the first genetic locus associated with NIDDM, in Fiji Islanders who were descended from North Indian migrants. This group has also investigated the relative contributions of obesity, ethnicity, and urban/rural residence to the prevalence of NIDDM. For example, the prevalence of diabetes in urban Polynesians in Western Samoa is almost three times the prevalence in rural Polynesians on that island, implicating an environmental effect.

On Loyalty Island, urban Melanesians have only about one-third the rate of diabetes of urban Polynesians, implicating a genetic effect. Further, diabetes is rare in both Polynesian and Micronesian populations which have maintained a traditional lifestyle, suggesting a socio-cultural basis to the onset of diabetes.

Clinical Trials Program

The plans formulated during FY 1981 for a multicenter randomized clinical trial to assess the relationship between blood glucose control and the early vascular complications of insulin-dependent diabetes mellitus have been implemented during FY 1982. Twenty-one of the sixty-six responses to the Request for Application for clinical centers were funded in March 1982 as collaborative agreement grants--the first use of this new funding mechanism for a multicenter clinical trial. The collaborative agreement grant allows for the greater administrative flexibility needed for efficient management of this study and enables participation of program staff as full partners with the grantees in all aspects of the trial. The George Washington University Biostatistics Center was selected from among 14 proposals to be the coordinating center for the trial. This center was funded under the contract mechanism in early February 1982. The name for this study, selected by majority vote of the participants, is the Diabetes Control and Complications Trial (DCCT).

A Steering Committee consisting of the Principal Investigators of each clinical center, the coordinating center, and two NIADDK staff members was convened for the first time under the chairmanship of Dr. Oscar Crofford of Vanderbilt University on February 19, 1982. Since then it has met on a monthly basis. Four working committees have been established which report to the Steering Committee through a Planning Committee. The Planning Committee includes working committee chairmen, two NIADDK staff, two representatives of the coordinating center, and the chairman of the Steering Committee. The four working committees are: Treatment, Eligibility, Standards and Methods for Metabolic Assessment, and Complications. These committees are composed of Principal Investigators and others from the participating clinical centers. In addition, consultative expertise from individuals not participating in the study is utilized as needed. This committee structure is working toward the development of a completed protocol by August 1982 and a detailed Manual of Operations (including study forms) by December 1982. A two-year feasibility study involving approximately 250 patients (Phase II) is planned to begin in February 1983.

A Data, Safety, and Quality Review Group (DSQ) will review the protocol for Phase II before implementation and will monitor the data as Phase II proceeds. The DSQ will be comprised of experts in diabetes, biostatistics, and clinical trial methodology who are not otherwise involved with the trial. In addition, a Policy Advisory Group (PAG), with a membership more broadly representative of the diabetes community, will approve the protocol proposed for Phase II as well as review progress of the trial at major decision points. In particular, the PAG will have a significant role in the key decision to be made toward the end of Phase II as to whether or not to proceed with Phase III, the definitive study to be conducted with a much larger number of subjects over a period of 7 to 10 years. The present schedule calls for Phase III to begin early in 1985.

The success of this program is heavily dependent on predictable and stable funding. The nature of the existing annual budget process is not entirely in harmony with this need, which calls both for long-range planning and long-term commitment of support by the Institute.

SPECIAL PROGRAMS BRANCH

This Branch administers the Division's Diabetes Centers Program, National Diabetes Data Group, and National Diabetes Information Clearinghouse. During the past year, the Division's National Research Service Awards and Career Awards have also been administered by staff of the Special Programs Branch.

Diabetes Centers Program

Background. This program provides support for both Diabetes-Endocrinology Research Centers (DERC) and Diabetes Research and Training Centers (DRTC). The primary requirement for establishment of either a DERC or a DRTC is a strong base of high-quality, ongoing biomedical research. DRTCs have, in addition to a research component, a training/information transfer component which includes training of both medical and allied health professionals, continuing education, model demonstration, and outreach activities. For both types of centers, the grant provides for core facilities (shared resources), pilot and feasibility studies (new initiatives), and limited funds for program enrichment. This funding is intended to strengthen existing programs, increase productivity, and foster the generation of new ideas and innovative research approaches. Although each center is expected to develop its own independent program in accordance with local needs, interests, and resources, each is also expected to be responsive to national needs, and must be willing to work cooperatively with the NIADDK and, in the case of DRTCs, with other Federally-supported diabetes programs.

Presently Funded Centers.

During the past year, the program provided support for 4 DERCs and 7 DRTCs as follows: Diabetes Endocrinology Research Centers - University of Pennsylvania (Philadelphia), University of Washington (Seattle), University of Iowa (Iowa City), Baylor School of Medicine (Houston); Diabetes Research and Training Centers - Albert Einstein College of Medicine (Bronx), Indiana University (Indianapolis), University of Michigan (Ann Arbor), Washington University* (St. Louis), University of Chicago* (Chicago), Vanderbilt University* (Nashville), University of Virginia* (Charlottesville).

A mechanism for support has also been developed which allows DERCs the opportunity to become DRTCs. A DERC may apply for a modest exploratory grant to develop the resources necessary for initiation of DRTC training and information transfer activities.

Research Activities at the Centers.

The vast majority of funding of research at the centers comes from NIH R01 and P01 (or equivalent) grants to center investigators. However, the concept of shared biomedical resources provided by the center funding allows for enhanced productivity and increased multidisciplinary efforts. An excellent example of the success and value of biomedical cores is illustrated by the research on pancreatic transplantation at the Washington University Diabetes Research and Training Center in St. Louis. This research group has expanded their various studies related to transplantation of pancreatic islet cells and has made available this resource to investigators from several other laboratories and sections of the medical school representing different scientific disciplines. This research on pancreatic transplantation has been highly productive and of high quality as reflected in the fact that one of the participating investigators was the recipient of the "3M" Life Sciences award by the Federation of American Societies for Experimental Biology in 1981. Three of the core facilities (Radioimmunoassay, Morphology, and Tissue Culture) contributed significantly to this work.

A group of investigators from different scientific disciplines at the University of Chicago-Michael Reese Hospital Diabetes Research and Training Center have collaborated over the past few years to significantly advance our understanding of how insulin is synthesized and secreted from the pancreas, as well as elucidating the structure of insulin and its precursor. This work is being continued on the pancreatic hormones, glucagon and somatostatin, and pancreatic polypeptide, utilizing sophisticated cell sorting instrumentation available in the centers' Tissue Culture core and highly advanced techniques available from the

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*Previously supported as a DERC.

Peptide Purification core. The basic research being conducted with the support of these cores is continually being extended into the clinical area. For example, during the past year, a collaborative study between the basic scientists in the Department of Biochemistry and clinical investigators in the Department of Obstetrics and Gynecology, provided evidence that levels of a fragment (C-peptide) of the precursor molecule of insulin (proinsulin) in the amniotic fluid of pregnant women provides an accurate means of assessing secretion of insulin from the fetal pancreas in utero. These investigators also found that amniotic fluid C-peptide levels are a reliable indication of intrauterine growth, and their data suggest that insulin production is directly related to observed differences in growth rates of fetuses. Determinations of C-peptide levels by the Radioimmunoassay core played a significant part in this study at the University of Chicago.

The Animal Resources Core at the Vanderbilt University Diabetes Research and Training Center provides an excellent example of diabetes animal model cores. This core provides assistance, space, and equipment to center investigators who are conducting diabetes-related research on canine models of diabetes. The core provides animal care and monitoring, training of new investigators in basic surgical and experimental procedures, and development of new models for diabetes-related research in conscious dogs. The availability of an experienced surgical technician to prepare animal models consistently and reliably, and to teach animal surgical techniques has significantly reduced variability in this type of research as well as overall costs to individual investigators. This core facility is used for diabetes-related research by investigators from several departments and scientific disciplines. The use of the dog models has increased since the establishment of the core, and many investigators who would otherwise not have been able to do so are able to utilize these models. At a recent site visit for renewal, the reviewers concluded that the quality of research utilizing the dog models has been excellent and that this facility provided a major resource for the center investigators. The Director of this core facility at Vanderbilt University has demonstrated the techniques and services provided by the core to two other Diabetes Research and Training Centers.

Pilot and feasibility studies are another aspect of the research component of centers. The center awards provide modest support for three years or less to allow researchers to explore the feasibility of a concept and collect sufficient data to apply for independent funding. This mechanism provides a unique opportunity not only for testing new ideas, but also for young investigators and established investigators in other disciplines to apply their expertise in the areas of diabetes. Thus far, the majority of investigators receiving pilot and feasibility funding have evidenced continued interest in diabetes research as indicated by being approved for continued funding from NIH or other peer-reviewed support by approximately 40% of the investigators.

Two pilot and feasibility studies at the University of Pennsylvania have had particularly unique aspects. The first, involving a noted histopathologist in the diabetes area and a leading molecular biologist, has continued to flourish over a number of years beyond the funding of the pilot and feasibility study. The second brought together an established investigator in diabetes research with an expert in computer simulation to test the feasibility of applying computer techniques to construct a model of glycolysis in pancreatic beta cells. This model, based on information concerning the properties of individual enzymes in the glylcolytic pathway determined in vitro, will be compared with the observed insight into adaptive control mechanisms in finite structures. Both of these instances represent the initiation of an interdisciplinary effort which might not have otherwise been possible.

The centers have also been instrumental in undertaking studies on the psychosocial problems of the diabetic patient, a heretofore neglected area of research. The University of Virginia DRTC has two pilot and feasibility studies investigating methods for improving patient compliance. The Vanderbilt, Washington University, and Einstein-Montefiore DRTCs are also supporting work in this area.

Activities of the Training and Information Transfer Component at the DRTCs. Prior to establishment of the DRTCs, each of the center institutions provided training and educational experiences based primarily on traditional didactic methods. With the establishment of the DRTC program, centers have been able to enlist the aid and expertise of specialists with knowledge and experience in the most contemporary methods of health education and evaluation. This has enabled centers to develop and test innovative and effective ways of approaching training and education of health professionals concerned with the diabetic patient.

Although biomedical research is a primary focus at the DRTCs, the long range goals of these centers include the transfer of this knowledge base from the research laboratory to the wide spectrum of health professionals engaged in the care, education, and management of the diabetic patient. This includes development of a national resource of programs which provide excellent and effective training of health professionals on the most current methods of diagnosis, management, and treatment of diabetes and its complications. Developed and tested training and information transfer activities are then disseminated to a broader spectrum of health professionals. Thus, the product of this center component is development of "exportable" programs for training health professionals.

All of the DRTCs are involved in the development and evaluation of materials for continuing education programs related to the care and treatment of the diabetic patient. Once the materials developed have been evaluated, every effort is made to have them deposited with the National Diabetes Information Clearinghouse to ensure the greatest possible dissemination. The "team approach" to care and treatment of diabetic patients is an aspect that is being explored and utilized to some extent at all of the DRTCs. This aspect represents an initial focus by the Einstein-Montefiore DRTC, and represents a significant accomplishment by this center. Their multidisciplinary Diabetes Education Team provides teaching, evaluation, and consultation services to both its parent medical center and local agencies. Comprised of an internist, social worker, nutritionist, nurse, and podiatrist, the Diabetes Education Team has undertaken a variety of activities that make it the focal point of training and information transfer at this center. This team, by giving equal importance to the psychosocial and biomedical aspects of diabetes, serves as a "model" not only for the education of the health professional, but also for the diabetic patient. Training programs for teams at other medical facilities have also been initiated.

The training and information transfer components of DRTCs also have an impact on medical education in their host institutions. For example, the Vanderbilt DRTC offers fourth year medical students a clinical elective in diabetes which differs substantially from the traditional curriculum. Students observe and work in the DRTC Model Demonstration Unit two half days a week for four weeks. Student time is divided between lectures, participation in the care of both adult and pediatric patients, and case discussions with the health care team. Medical students attend lectures given by different health professionals, including nurses, dietitians, clinical psychologists, and physicians. Nursing, dietary, psychology, and education students also attend the medical student lecture series, enhancing the team approach to the care of the diabetic patient.

The transfer of useful approaches to diabetic patient care is enhanced by developing relationships between the centers and other Federal agencies which support diabetes-related programs. DRTCs and nearby CDC Diabetes Control Projects have been exploring possible collaborative efforts and ways to exchange information. The centers also cooperate with the Health Services Administration's Indian Health Service (IHS) Diabetes Program. The IHS Diabetes Program is continuing to determine the feasibility of providing education and training on diabetes to Indian Health Service personnel at five selected locations.

Arrangements among the Indian Health Service, the NIADDK, and the DRTCs have been made which will allow the Director of this program to utilize some center personnel in conducting these training programs. This experience is valuable to the DRTCs in evaluating the effectiveness of certain training programs in populations and cultures otherwise not available to the centers. This collaboration also benefits the IHS program by providing needed medical expertise in diabetes for its training program.

Thus, the DRTCs offer a unique resource for interaction between traditional biomedical research and health care delivery activities and are clearly excellent places for the development of programs which reflect the latest research advances applied to training and continuing education for health professionals. However, the widespread application of these programs is clearly outside the mandate of the NIH. Therefore, funding for the application of such programs must come from the other Federal, State, and private agencies with interests and/or responsibilities in health care delivery. Realization of the full potential of the DRTCs in the future will depend on the full cooperation and support of other Federal agencies through their health care delivery programs and activities.

National Diabetes Data Group

The Data Group serves to foster, coordinate, and integrate national resources for the collection and analysis of data on diabetes in order to provide a sound basis for the development of scientific priorities and the planning of public health programs. The Data Group also continues to provide the Federal focus to which both the lay and scientific communities turn for accurate, reliable statistics on diabetes.

The National Diabetes Data Group consists of a nationwide group of expert advisors including epidemiologists, individuals with expertise in the biomedical, nutritional, and socioeconomic aspects of diabetes, and representatives of Federal agencies (the National Institutes of Health, the Centers for Disease Control, the National Center for Health Statistics, and the National Diabetes Advisory Board) and the private sector (the American Diabetes Association and the Juvenile Diabetes Foundation).

The Data Group held three meetings during FY82 and is embarking on three new major projects: (1) achievement of an international consensus on methodology for measurement of glycosylated hemoglobin and its use in clinical practice, research, and screening for diabetes; (2) assessment of the economic methodologies for measuring cost of disease and generation of accurate data on the economic impact of diabetes and its complications; and (3) development of a comprehensive data book on diabetes to respond to the data needs of researchers, public health officials, clinicians, and other persons and agencies concerned with diabetes.

The Data Group continues to work in close collaboration with the National Center for Health Statistics in assessing the national data on diabetes collected by that agency. With the Division of Health Examination Statistics, the Data Group is analyzing oral glucose tolerance tests on a statistical sample of the U.S. population to develop norms and standards for plasma glucose levels and to determine the prevalence of undiagnosed diabetes and impaired glucose tolerance. This Division will also be conducting a study of Hispanic-Americans, for which the Data Group developed the medical history and household questionnaire, and the clinical and lab tests to be used. An analysis of the changing prevalence and incidence of diabetes over the last forty years was completed in collaboration with the Division of Health Interview Statistics.

The Data Group is also collaborating with the National Diabetes Information Clearinghouse in analyses of data collected through a special diabetes questionnaire which was a supplement to the 1976 Health Interview Survey. These analyses include quantification of the rate of diabetes among parents and siblings of diabetics, borderline diabetics and non-diabetics; the prevalence of cardiovascular risk factors among diabetics, including hypertension, obesity, and smoking; the prevalence of macrovascular and microvascular disease; history of large birth weight babies; and the rate of diabetes among defined age/income/education groups.

These analyses were expanded to include assessment of the medical care aspects of diabetes contained in the National Ambulatory Medical Care Survey and the National Hospital Discharge Survey.

National Diabetes Information Clearinghouse

The National Diabetes Information Clearinghouse (NDIC), entering its fourth year of operation, continues to be the primary source of information to health care providers, people with diabetes, medical writers, and the general public about diabetes, materials, programs, and outreach efforts. As a means of examining the existing program and to aid in designing an evaluation, an evaluability assessment (EA) was conducted in FY 82. The results of this EA have provided a number of management options. Additionally, performance indicators which will assist in future evaluations were identified for each potential NDIC activity. The EA report will be used by the clearinghouse program director and the advisory committee in determining the major foci of the program for the next three years. A related evaluation effort now in progress will assist the program director in designing an effective and continous performance monitoring system. One of the specific tasks of this project is to evaluate the utility of the NDIC annotated bibliographies. The results of this evaluation and an outline for a performance monitoring system for all NDIC activities will be available in late summer.

NDIC staff have continued to actively solicit diabetes materials and descriptions of programs and have entered over 3,300 abstracts in an automated data system. Cooperative activities with voluntary organizations, other NIH Institutes, and other Federal agencies are also continuing. The NDIC has provided partial financial support for conferences supported by the Juvenile Diabetes Foundation, the National Diabetes Research Interchange, and the National Diabetes Data Group. The NDIC also funded an evaluation of materials designed for the Native American population living in the Southwest.

Diabetes Dateline will continue to be published while OMB considers a request for exemption to the printing moratorium. A request for exemption has also been submitted to OMB for a revision of one of the most requested annotated bibliographies, Educational Materials for and About Young People With Diabetes. Currently, only one future publication, A Glossary of Diabetes Terms, has a prior clearance which will allow publication. This Glossary is intended for the newly diagnosed person with diabetes and the definitions are written to provide brief, simple descriptions that can be understood by a person with an 8 to 10th grade education.

National Research Service Awards (NRSA)

The mission of the Training Program (NRSA support) is to provide research training for the most promising scientific investigators in diabetes, endocrinology, and metabolic diseases. The number of applicants remains high and approximately 334 slots (both individual and institutional) currently are being funded. One reason for the increased number over last year's levels is the decrease in institutional allowances. This decrease, coupled with an increased funding level, has provided the program with a significantly greater ability to fund meritorious new and competing applications.

Research Career Development Awards

The Research Career Development Program continues to pursue its mission of attempting to assure an adequate supply of well-trained investigators in the areas of diabetes, endocrinology, and metabolic diseases. During FY82, 28 new career awards were made to promising investigators in these three fields. The Special Emphasis Research Career Program in Diabetes, jointly sponsored with NHLBI, NIA, and NICHD, has been continued and seven of the new career awards were made to SERCA applicants. Discussions were held with NINCDS regarding possible expansion of the SERCA to include neurologic aspects of diabetes and with NIAID regarding the inclusion of immunologic aspects.

ENDOCRINOLOGY AND METABOLIC DISEASES PROGRAMS BRANCH

This branch administers the Endocrinology and Metabolic Diseases Research Programs of the Division. In addition, the office of the Branch Chief serves as the focus for planning and implementing special initiatives and for administering NIADDK cystic fibrosis-related activities.

Endocrinology Research Program

The Endocrinology Research Program supports the research of approximately 250 investigators. The program's major emphasis is on basic research into the mechanisms of hormone action, hormonal control mechanisms, and synthesis of hormones. A smaller but significant portion of the program effort goes into clinical research. Studies of thyroid, parathyroid, adrenal, pituitary, thymus, and pineal hormones are supported, as well as studies on the neuropeptides and prostaglandins. During the past year there was a change in program emphasis such that projects investigating the basic structure of peptide hormones were shifted to the Metabolic Diseases Program while projects concerned with insulin and/or glucagon were moved to the Diabetes Program. Some recent advances supported by the Endocrinology Research Program are summarized below.

Adrenal Medulla

The adrenal medulla is actually a portion of the sympathetic nervous system, but is considered to be an endocrine organ because it secretes epinephrine and norepinephrine. These are hormones which have systemic effects as well as local effects in that they act as neurotransmitters at nerve endings where they are also produced.

Pheochromocytomas are tumors of the sympathetic nervous system usually found in the adrenal medulla. Among other symptoms, they cause persistent hypertension. Their presence may be diagnosed by elevated levels of epinephrine and norepinephrine or their derivatives in plasma and urine. Some pheochromocytomas develop in areas other than the adrenal medulla and are often difficult to locate for surgical removal, although their presence is known by their abnormal hormone production.

A researcher whose work is supported by the Endocrinology Program has developed a scintigraphic method for locating these tumors. He injects a radio-labeled analogue of guanethidine called [^{131}I] meta iodobenzyl guanidine ([^{131}] MIBG). This material resembles norepinephrine in its molecular structure, and appears to enter adrenergic tissue just as do the natural hormones. The MIBG is injected 24 hours or more before a scan is made. The MIBG concentrates in tissues having adrenergic nerve endings, but it does not remain there more than a few hours. On the other hand, tissues with large reservoirs of adrenergic hormones would be expected to maintain their concentration of radio-labeled MIBG for longer periods of time. Thus, tumors which are producing abnormally large quantities of adrenergic hormones concentrate MIBG and may be detected by the scanning methods used.

The method has successfully detected the presence of tumors which were not visualized by other methods, thus aiding surgeons in finding them for removal. The method appears to be sensitive and has no detectable adverse side effects. This advance is of particular interest because of its direct application to clinical medicine.

Adrenal Cortex

So called "essential hypertension" or high blood pressure is a common disease whose etiology is at present unknown. However, it is suspected that the adrenal cortex in some manner is responsible. One of the observations in hypertension is that there is a retention of sodium by the body, and an adrenal salt retaining factor is known to exist. Its identity is unknown, except that it is known to be a mineralocorticoid other than aldosterone.

Following adrenal enucleation (i.e. removal of the adrenal medulla), rats are in positive sodium balance and have impaired ability to excrete a sodium load. By incubating regenerating enucleated rat adrenals, researchers have been able to show that a compound called 19 hydroxy deoxycorticosterone (19-Hydroxy-DOC) is produced by the glands during the early sodium retention phase. At about the same time another researcher was able to show that a different compound, 19-nor-DOC which is a potent mineralocorticoid could be isolated from the urine of adrenal enucleated rats. Following a series of experiments, it was concluded that 19-hydroxy-DOC is probably the precursor of 19-nor-DOC and 19-nor-DOC may be the mineralocorticoid responsible for sodium retention. Further studies showed that 19-nor-DOC production is markedly enhanced by the administration of ACTH.

As a result of the above observations, studies were initiated in normal and hypertensive human subjects to determine the patterns of 19-nor-DOC excretion in the urine. These studies indicate that ACTH is a major activator of 19-nor-DOC production in humans. The investigators suggest that 19-nor-DOC may be important in the regulation of systemic arterial pressure and that it deserves intensive further study in normal subjects and in human hypertensives.

Corticotropin Releasing Factor (CRF)

Recently a group supported by the program announced the isolation and characterization of CRF, thus ending a search of over 20 years for this hypothalamic peptide. Since the purification and synthesis of CRF, various studies have shown that it causes the release of ACTH and B Endorphin from the pituitary in experimental animals and in man. Other studies are now in progress to determine whether CRF also has direct effects on various peripheral tissues. The synthesis of CRF therefore provides a powerful new tool for defining precisely how the central nervous system regulates the body's response to stress. New insights in this area eventually may lead to improved therapeutic interventions for various stress-related diseases including peptic ulcer, heart disease, stroke, and sexual dysfunction.

Parathyroid Hormone (PTH)

Investigators are studying the factors which influence the secretion of parathyroid hormone, one of the hormones regulating calcium balance in the body.

Investigators using an in vitro preparation of bovine parathyroid gland slices have been able to measure the effects of changes in the concentration of extracellular calcium on cellular levels of parathyroid hormone messenger RNA. Low extracellular calcium levels cause the production and secretion of PTH, whereas high extracellular levels of calcium cause suppression of PTH secretion. It has been demonstrated that total hybridizable PTH mRNA did not change as the calcium levels were increased, but that polyadenylate rich PTH mRNA increased 2 to 3 fold over a period of 5 to 7 hours. It has also been determined that PTH mRNA is very stable and that the transcription rate of the PTH gene is very low.

These results suggest that short-term regulation of secretion of PTH takes place at a post-transcriptional level in the cellular manufacture of parathyroid hormone. More specifically, this short-term regulation occurs at the stage beyond where the message from the gene has been transcribed, but before it has been translated into the actual protein (hormone). This short-term control appears to take place by modification of the polyadenylate portion of the mRNA, a change in this portion either speeding up or slowing down the speed of translation to the hormone. These results also support another possibility, that the long-term regulation of secretion of hormone by the parathyroid may occur mainly by cell proliferation, rather than by increased production of hormone from the already existing cells. Further work along these lines is in progress.

Thyroid Studies

It has been known for some time that the active thyroid hormones, T_4 and T_3, do not cross the placenta from the pregnant mother to the fetus and that the fetus produces its own thyroid hormones. However, recent results shed additional light on the exact role of the placenta relative to these hormones. Studies have shown that homogenates of placenta deiodinate T_4 and T_3, converting them to various inactive iodotyrosines. This suggests that the placenta may function not as a simple barrier per se to the mother's thyroid hormones but rather as a site for conversion to inactive compounds which then may or may not cross the placenta to the fetus.

Thyroid hormone appears to act in concert with other hormones. Recent studies have been designed to determine the effect of thyroid hormone on uterine estrogen and progesterone receptor levels and the uterine receptor response to estradiol action. In this regard, immature female rats were first ovariectomized and made hypothyroid by chemical methods. Estrogen receptors in the cell sap and nucleus were measured as was cell sap progesterone, both before and after 5 day replacement therapy with triiodothyronine. The results showed significant uterine weight gains and increase in cell sap estrogen and progesterone receptors of T_3 treated animals over the ovariectomized, hypothyroid controls. These studies suggest that thyroid hormone affects the uterine sensitivity to estrogen by modulating the availability and replenishment of cell sap estrogen receptors. In addition, they also suggest a potential mechanism for the regulation of uterine sensitivity to progesterone action.

Lymphocytic infiltration and destruction of the pancreatic beta cells results in spontaneous autoimmune insulin-dependent diabetes mellitus in approximately 30% of BB/W rats at 60 to 120 days of age. Morphologically and physiologically this syndrome in the BB/W rat resembles Type I insulin-dependent diabetes mellitus in man. In view of the increased frequency of autoimmune chronic thyroiditis (Hashimoto's) in patients with diabetes, studies were carried out to determine the incidence of lymphocytic thyroiditis in diabetic and non-diabetic BB/W rats aged 8 to 10 months. The results were as follows: there was no evidence of lymphocytic thyroiditis in non-diabetic rats. In contrast, thyroiditis occurred in one-half of the diabetic rats. Serum T_4 and TSH concentrations were similar in non-diabetic and diabetic rats, irrespective of the occurrence of thyroiditis. It is well known that excess iodide ingestion induces hypothyroidism in patients with Hashimoto's thyroiditis and it has been suggested that excess iodide in the diet may be partially responsible for the apparent increased incidence of this disorder. Studies were therefore initiated to determine whether the ingestion of excess iodide would increase the incidence of thyroiditis in non-diabetic and diabetic BB/W rats or induce hypothyroidism in diabetic rats with lymphocytic thyroiditis. The iodide treatment induced mild thyroiditis in a few of the non-diabetic rats and increased both the incidence and intensity of the thyroiditis in the diabetic rats. The conclusion from these studies is that spontaneous lymphocytic thyroiditis occurs frequently in diabetic BB/W rats suggesting a close genetic linkage between these two autoimmune diseases. Excess iodide ingestion induced mild thyroiditis in 32% of the non-diabetic rats and increased the frequency and intensity of thyroiditis in diabetic rats. These latter findings suggest a possible role of excess iodide ingestion in the pathogenesis of lymphocytic thyroiditis.

Pituitary Gland - Target Organ
TSH - Thyroid Studies.

Thyroid Stimulating Hormone (TSH) from the anterior pituitary stimulates the thyroid gland to produce more thyroid hormone. It has been suggested that the effects of TSH on thyroid metabolism may be mediated by the activation of an adenylate cyclase cyclic AMP second messenger system. However, TSH seems to remain bound to thyroid tissue, so the mechanisms which control or terminate the actions of TSH on the thyroid are not well understood. It is possible that desensitization or refractoriness of the cell to hormonal stimulation following previous exposure to the hormone might be responsible for the physiologic regulation of the target cell sensitivity.

Previous experiments have shown that rats with chronically high endogenous TSH levels have a decreased ability to produce cyclic AMP by stimulation from TSH. Thyroids taken from hypophysectomized rats however, seem to produce more cyclic AMP when TSH is given. A series of experiments was developed to determine how thyroid tissue responds to TSH.

Cyclic AMP concentrations were measured in thyroids taken from normal rats and those from hypophysectomized rats. Each group of thyroids were given 3 doses of TSH. Hypophysectomy caused a decrease in the basal concentration of cyclic AMP. TSH, in varying doses, caused an increase in cyclic AMP in thyroids from hypophysectomized rats.

The number of receptor sites for TSH was determined for the thyroid cells of both normal and hypophysectomized rats. It was determined that the number of TSH binding sites was significantly increased in membrane preparations from thyroids of hypophysectomized rats as compared to controls.

The results are interpreted as follows:

1) Hypophysectomy results in a significantly increased response of the cyclic AMP system to TSH stimulation.

2) Hypophysectomy causes an increase in the number of TSH receptors in thyroid cell membranes. This suggests that the hyperresponsive cyclic AMP system in hypophysectomized rats is a result of an increased number of TSH receptors. Other workers have shown that high endogenous TSH levels cause a decrease in the number of TSH receptors. Thus, it would appear that endogenous TSH inversely regulates the number of its own receptors on thyroid cells.

Thyrotropin releasing hormone (TRH), which stimulates production of TSH in the pituitary, was the first of the hypothalamic releasing factors to be characterized. Recent studies have shown that it also has the ability to increase prolactin production in the pituitary gland. In this regard, it appears that TRH stimulates prolactin production by first increasing the messenger RNA which in turn causes an increase in prolactin biosynthesis. Since the data indicate that the TRH actually seems to stimulate production of the precursors of mRNA, the conclusion drawn is that this small polypeptide hormone (3 amino acids) acts directly on the genetic material promoting transcription of the prolactin gene.

Growth Hormone

A smaller molecular variant of human Growth Hormone known as hGH-20K has been studied because it exhibits properties somewhat different from the usual hGH. It has been found that hGH-20K stimulates both growth in the rat and somatomedin production *in vivo*. However, it is inactive as a diabetogenic substance in that it does not cause glucose intolerance or hyperinsulinemia in the dog. Limited proteolytic digestion of the molecule did not generate diabetogenic activity, although it does do so with regular hGH. This variant has been found in some supposedly normal human pituitaries, and thus it is considered to be a part of the "family" of pituitary growth hormones.

The pituitary gland regulates fat mobilization and the mass of the adipose organ of the body by three mechanisms: a) the ACTH/alpha MSH/beta MSH group of pituitary hormones; b) pituitary growth hormone; and c) the catecholamines--mainly epinephrine. Four different advances have been made this past year relating to fat mobilization.

1. It has been demonstrated for the first time in humans that the production of epinephrine depends on endogenous ACTH. Children with ACTH deficiency were shown to be profoundly hypo-epinephrinemic even after exercise. Correlated with this defect were subnormal free fatty acids, subnormal basal metabolic rates, and increased adipose mass.

2. The role of growth hormone in restricting adipose mass was also demonstrated in human subjects. In a study of 50 short children being evaluated for growth failure, endogenous GH function was measured by radioimmunoassay and adipose mass was quantified anthropometrically. Adipose mass proved to be inversely related to GH availability. The measurement of adipose mass was shown to be a useful rapid screening method for identifying the GH-deficient short child.

3. With progressive age in humans, the lean body mass declines and the adipose mass expands. A possible underlying endocrine mechanism was identified by showing that endogenous GH secretion declines progressively in healthy adults after age 40. By age 60, about one half of the general population is GH deficient.

4. The ACTH/alpha MSH/beta MSH group of peptides share the following active amino acid sequence: tyr ser met glu his phe arg. This active site appears to endow all three peptides with lipolytic and melanotropic properties. Previously, only ACTH among the three was believed to possess corticotropic activity. However, it has recently been demonstrated that during fetal life alpha- and beta-MSH both possess corticotropic activity. The data suggest that these two peptides may influence the embryonic growth of the adrenal cortex during fetal development.

Hormone Distribution

During the past year, Requests for Proposals for hormone extraction, purification, and distribution were advertised and new contracts were negotiated. The University of Maryland was selected to continue collection of human pituitaries and to distribute hormones extracted from them to qualified investigators. This program, formerly the National Pituitary Agency, is now known as the National Hormone and Pituitary Program (NHPP). Other materials distributed by the NHPP include animal pituitary hormones and antisera to human and animal hormones, Relaxin, and Chorionic Gonadotropin. Both the NICHD and the USDA contribute materials and financial support to the NHPP.

For approximately 20 years, the NHPP has distributed its material free of charge to qualified investigators who submit an acceptable research protocol. As of July 1, 1982, this policy was modified to provide that recipients assume the costs of handling and shipping hormones and antisera.

There has also been continued progress related to the production of human Growth Hormone by genetically-modified bacteria. Much of the basic research underlying this development was supported by the Endocrinology Program and reported in previous years. Bacterially-produced hGH is now being manufactured by a commercial company. Published reports show that the bacterial hGH causes growth in test animals that is indistinguishable from growth caused by natural hGH. In addition, clinical trials in humans have been initiated. The NHPP is collaborating with the private sector in this important project by supplying natural hGH to use as control material for tests on the purity and antigenicity of bacterial hGH. If and when bacterial hGH becomes available for routine use, not only will there be a fully adequate supply available to treat short-stature children, but it should also be available for use in other clinical studies involving accelerated healing of bone fractures, burns, ulcers, etc.

Metabolic Diseases Research Program

The primary objectives of this program are to support investigator initiated studies of basic metabolic processes and basic and clinical studies of various inborn metabolic diseases (e.g., cystic fibrosis, lysosomal storage diseases, glycogen storage diseases, etc.). Major foci of basic research investigations include membrane structure, biological transport, and enzymatic mechanisms as they regulate normal and abnormal metabolic processes. Additional areas of emphasis include studies related to the diagnosis, etiology, pathogenesis and treatment of a large number of inborn metabolic diseases. Some highlights of research accomplishments during the past year are summarized below.

Diagnosis and Prevention of Inborn Errors of Metabolism

General Background. Inborn errors of metabolism are characterized by enzymatic defects affecting metabolic transformation. The lack of sufficient enzymatic activity results in the interruption of the normal metabolic process and causes, in most cases, accumulation of unneeded, toxic intermediates. Sometimes, the enzymatic defect is due to structural changes in the protein, resulting from gene mutations. Other times, the absence of enzymatic activity is due to the lack of a factor (e.g., a vitamin) needed to activate the enzyme. In both cases, the earliest possible correction of the enzyme deficiency could correct the affected metabolic process and thus eliminate or reduce the severe sequella of the deficiency. In many cases, early identification of carriers of these diseases appears to be the most practical approach to prevent or significantly reduce occurrence of the disease.

Prenatal Diagnosis and Treatment of Biotin Dependent Multiple Carboxylase Deficiency.

A significant advance in the prevention and treatment of hereditary metabolic diseases is illustrated by the recent clinical application of new knowledge regarding the nature of a specific biotin (vitamin H) dependent, multiple carboxylase deficiency. Following biochemical characterization of this disorder in a young child, a prenatal diagnosis was made in a second child through amniocentesis. Administration of biotin to the mother during the second pregnancy prevented subsequent manifestation of this disorder in the newborn, demonstrating the usefulness of amniocentesis for both prenatal diagnostic and therapeutic procedures.

Carrier Identification in Maple Syrup Urine Disease.

Defective functioning of the enzyme BCKA dehydrogenase causes *in vivo* accumulation of intermediates formed during metabolic degradation of three amino acids (valine, leucine, and isoleucine), which are components of dietary proteins. The resulting Maple Syrup Urine Disease (MSUD) is an inherited metabolic disorder leading to mental retardation and retarded motor development in afflicted infants. Unfortunately, carriers of the mutant gene, whose children could be afflicted with MSUD, could not previously be identified and thus genetic counselling has not been possible. Recent progress, however, has resulted in the development of an assay for BCKA dehydrogenase in disrupted cultured fibroblast (skin) cells. Investigators found that disrupted fibroblasts from obligate carriers showed a different BCKA dehydrogenase activity pattern than cells cultured from normal and diseased individuals. Further validation of the method is underway. The availability of this assay could result in broad scale prevention of this devastating disease.

In vivo Activation of a Defective Enzyme.

A means of correcting or reactivating defective enzymes through oral or intravenous administration of various chemical agents recently has been developed. This novel therapeutic strategy has been evaluated in a cat model of human mucopolysaccharidosis VI, a lysosomal storage disease which is due to a lack of the enzyme arylsulfatase-B. The two subunits of the normal cat enzyme are bound together, while in the mutant enzyme they are separate and inactive. Chemical manipulations by means of cysteamine reunited the two subunits and activated the mutant enzyme. Such research results suggest strategies for the targeted delivery of therapeutic agents to specific disease sites and the possibility of in vivo reactivation of mutant enzymes.

Hormones, Receptors, and Their Mechanism of Action

General Background. Receptors are proteins with specific binding sites for hormones. They help transfer the hormonal message to the target component of the cell. Two receptor systems are singled out for consideration: First, the adenylate cyclase system contains a receptor which binds various hormones (e.g., the beta-adrenergic receptor binds norepinephrine, epinephrine and isoproterenol, and regulates contractions of the smooth muscles). Second, the light-regulated phosphodiesterase system contains light-sensitive rhodopsin which activates the enzymes involved in visual processes occurring on the retina. Progress in understanding the nature of these two systems and their mechanism of action is described below.

Similarities Between the Adenylate Cyclase System (ACS) and the Light-Regulated Phosphodiesterase System (LRPS).

Lengthy research efforts indicated that the three major components of the ACS parallel similar components in the LRPS. There are: a) the ACS hormone receptor corresponds to rhodopsin of the photoreceptor; b) a protein called the G unit of the ACS corresponds to a GTP (guanosine triphosphate)-binding protein of the photoreceptor; and c) a catalytic moiety of the ACS corresponds to the enzyme phosphodiesterase (PDE).

To further study the similarities between AGS and LRPS, investigators performed a series of "cross" experiments between the two systems. They found that components from one system can interact with, and activate, the other system. In one set of experiments, illuminated rhodopsin, prepared from cattle and amphibian retina, was substituted for the hormone receptor complex of an ACS preparation from rat brain, containing active G unit and a catalytic component. The new system incorporated rhodopsin and acted as a true active ACS. In another set of experiments, ACS from brain synaptosomal preparations, hypothalamus membranes or frog erythrocyte ghosts, with G unit activity partially removed, was fully activated with GTP-binding protein purified from the photoreceptor.

The above substitution experiments argue for a remarkable degree of evolutionary and genetic conservation of mechanisms for intracellular communication.

Beta-Adrenergic Receptor and the Adenylate Cyclase System (ACS).

In recent years, the beta-adrenergic receptor from turkey erythrocyte membranes was isolated, solubilized and implanted into Friend erythroleukemia (Fe) cell membranes which were missing the receptor. The implanted receptor became coupled to the messenger system of the Fe cell membrane and the resulting ACS became active. While other components of the ACS were resolved, most recently investigators succeeded in locking a hormone in the beta-adrenergic receptor by a chemical attack on a reactive group associated with the active hormone-receptor complex. This result enabled the researchers to postulate a plausible mechanism for transmission of the hormonally stimulated message by the ACS. Moreover, due to the interchangeability of hormone receptors from different cells, the locking of the hormone by the method developed could provide a general way to study other adenylate cyclase systems.

Cystic Fibrosis (CF)

For several years, a number of investigators have attempted to develop procedures for detecting heterozygote carriers and prenatal tests for CF births. At a conference held in April, 1982, the data collected on these efforts were evaluated and it was determined that none of the approaches can be used currently in any but a strictly research context. Efforts in this area are continuing.

The past year has seen increased emphasis on exploring mechanisms of epithelial ion transport in normal and affected individuals. It has become increasingly clear that cystic fibrosis involves a defect in the reabsorption of sodium in sweat, salivary, and pancreatic ducts. Moreover, evidence points to the defect as residing in the passive transport of ions through the luminal membrane rather than being in the active transport of ions through the sodium pump (Na^+/K^+ATPase) in the basolateral membrane. In sweat and salivary ducts, sodium reabsorption appears to be decreased in CF patients while it may be enhanced in pancreatic ducts and respiratory mucosa. Increasing attention has been paid to the effects of the diuretic amiloride which, when applied to normal epithelial tissue, mimics some aspects of ion transport in CF-affected epithelia. Whatever mechanism is responsible for altered ion transport in CF, it may cause a quantitative difference rather than a qualitative one. A recent study of perfused sweat gland ducts showed that at very slow perfusion rates, sodium content of the sweat emerging from the duct is the same for normal and CF tissue, thus indicating that the transport defect may be one affecting rate rather than consisting of an absolute blockage of ionic flow.

THE ANNUAL REPORT OF THE DIVISION OF DIGESTIVE DISEASES AND NUTRITION

Harold P. Roth, M. D., Acting Associate Director

The Division of Digestive Diseases and Nutrition is responsible for the extramural support grant awards and contracts pertaining to nutrition and to diseases and disorders of the gastrointestinal tract and associated organs. Thus, digestive diseases include disorders of the esophagus, stomach, small and large intestines, liver, biliary tract and pancreas. Major digestive diseases include esophagitis, peptic ulcer, diverticulitis, infectious diarrhea, ileitis, ulcerative colitis, and malabsorption, gallstones, cirrhosis, hepatitis, and pancreatitis. The known causes include infections, metabolic disorders, genetic defects, neuromuscular disorders, toxins, and reactions to life stresses.

Digestive diseases constitute a health problem of great magnitude. Of all the causes of disability due to illness in our country, digestive diseases rank second. Over two million Americans are impaired in some degree by these diseases; 1.2 million persons are limited in the work that they can perform and 400,000 are disabled. Among the employed, digestive diseases each day account for 200,000 absences from work due to illness, making digestive diseases the leading cause of loss of time from work among male employees. Almost 140,000 veterans receive payments for service connected disability due to gastrointestinal conditions, at a cost to the nation of approximately 100 million dollars per year.

The Division of Digestive Diseases and Nutrition received more applications for research grants this fiscal year than it has received in any previous year. However, due to the constraints in funding, the number of research grants funded actually dimished from those in the previous year. Similarly, when the digestive diseases research center at Albert Einstein University applied for renewal, its support had to be diminished considerably below the recommended amount. Funds for the five clinical nutrition research units are also being reduced. The support for the other two centers in digestive diseases, the center for the study of liver diseases at the University of California at San Francisco, and the center for the study of peptic ulcer in Los Angeles were maintained. The principal investigator at the center for ulcer research in Los Angeles, Dr. Grossman, died and a new director, Dr. George Sachs, was selected.

There have been changes in the staff of the Division and their responsibilities. Marjorie Zukel, who had been our Administrative Officer, retired. Marsha Hennings, who had been the secretary to the Director, took her place. Mrs. Hennings in turn was replaced by Helen Abbott.

Dr. Richard Johannes, who joined the Division in July 1981 as an IPA from Johns Hopkins, has assumed responsibility for several of the Division's activities. One of these activities is the development of diagnostic criteria for functional bowel disease. He has worked with a nerve-gut committee established by the American Gastroenterological Association and chaired by Dr. Thomas Almy. This group held a scientific meeting to discuss the state-of-the-art under Dr. Almy's leadership during the year. Dr. Johannes is now planning a meeting to evaluate a medical interview designed to identify patients with functional bowel disease. It was first used by Dr. Grossman and Dr. Thompson. After the interview has been fully developed and evaluated by a meeting of experts, Dr. Johannes will apply it to a patient population in the Johns Hopkins Hospital out-patient department.

Dr. Johannes is also working on the study of the prevalance of gallstones in cooperation with the National Center for Health Statistics. The study is part of the Health Survey of Hispanic Americans. Dr. Johannes developed an interview which can be administered by physicians. This in turn can be compared with the responses to questions in the health survey as obtained by the regular interviewers. Dr. Johannes helped arrange a review of the efficiency and accuracy of the technicians who are performing the ultrasound studies for gallstones, and is working on plans to followup positive ultrasound studies with X-rays of the gallbladder. The preliminary and pilot studies have been completed and the definitive studies will begin in San Antonio. The interviews and questionnaires should make possible a comparison of the medical history and the findings of gallstones. Other data on medical history should be available in the data obtained from the national health survey.

Dr. Johannes is also working on the development of an epidemiology and data systems unit within the Division.

Dr. Donald Murphy, who also joined the Division late last year, is serving as Special Emphasis Coordinator for the Division. He has been organizing and preparing the reports of the Division, its records and its staff meetings, and making its plans, activities and policies available as appropriate. He has also served as Executive Secretary of the Digestive Diseases Interagency Coordinating Committee (DDICC). Dr. Murphy has been preparing a data system that will make possible prompt and accurate retrieval of information of the research activities of all institutes and agencies concerned with digestive diseases. The DDICC has been primarily concerned with an exchange of information. It is preparing an annual report under Dr. Murphy's direction, and it is cooperating closely with the Digestive Diseases Advisory Board. A member of the Digestive Diseases Advisory Board is now regularly attending meetings of the DDICC.

The National Digestive Diseases Advisory Board was established by Public Law 96-538 and chartered on May 13, 1981. The Board held its first meeting in November, 1981 and organized the following six subcommittees: Centers, Epidemiology and Impact, Research, Education and Information, Patient Care, and long-range Planning. The Board unaminously recommended the establishment and development of a broadly-based centers program for digestive diseases and recommended the development of an epidemiology and data systems program within NIADDK.

The Chairman and Deputy Chairman of the Board presented legislative testimony on behalf of these recommendations to the authorization and appropriations committees in the Congress.

The Board is now in the process of developing a program of activities in each of the subcommittees which is to evaluate and up-date the recommendations of the National Commission on Digestive Diseases. It is anticipated that the last meeting of the Board in FY 82 will be in July 1982 at which time a budget plan and an action plan for FY 83 will be adopted. The annual report for FY 82 for the Board will be available in July, 1982.

The Division is divided into four program areas, and includes responsibility for a clearinghouse. These five components, listed below, are reported in the subsequent pages:

PROGRAM	DIRECTOR
Esophageal, Gastric and Colonic Diseases	Dr. Kirt J. Vener
Liver and Biliary Tract Diseases	Dr. Sarah C. Kalser
Intestinal and Pancreatic Diseases	Dr. Gerassimos G. Roussos
Nutrition	Dr. Gerald F. Combs
National Digestive Diseases Education and Information Clearinghouse	Ms. Billie B. Mackey

ESOPHAGEAL, GASTRIC and COLONIC DISEASES PROGRAM

Kirt J. Vener, Ph.D., Program Director

The Esophageal, Gastric and Colonic Diseases Program area is responsible for the extramural support of grant awards and contracts pertaining to the listed organs. The following examples are representative of the progress in this area.

1. Advances in Esophageal Research The investigations which have been supported by NIADDK in the area of esophageal structure and function have focused on the general problems of esophagitis, the inability of the lower esophageal sphincter (LES) to maintain a tonus adequate to prevent the refulx of gastric contents into the esophagus (a common cause of esophagitis), and the regulation and integration of the propulsive muscular waves which move esophageal contents into the stomach.

Investigators at the Medical College of Wisconsin (Dodds, AM 25731) have attacked a problem which has confounded the measurement of the lower esophageal sphincter pressure (LESP). During the course of trying to measure LESP, it was clear that the axial movement of the esophagus caused by respiration and inspiration produced wide variances in experimental LESP measurements. Attempts to minimize this problem as a source of recording error have consisted of repeated repositioning of the manometric catheter, using multiple, closely spaced recording orifices, breath-holding during a rapid pull through, and pinning the LES to the catheter assembly in experimental animals. None of these techniques is satisfactory for long-interval, continuous recording of LESP in humans. A component was developed for fusing a special sleeve device for measuring LESP to the conventional catheter assembly. The sleeve device allows satisfactory continuous recording of LESP despite axial movement of the LES along the sleeve. The sleeve device has become an extremely useful research tool for certain types of investigative studies, particularly those dealing with gastroesophageal reflux.

Incorporating this modified pressure sensor with their catheter assembly in which pH could also be measured, the investigators were able to generate considerable information about the patterns of LESP and reflux which appear in patients with esophagitis as compared to controls. For example, the device was placed in the esophagus for 12 hour periods and the changes in LESP and changes in the frequency of reflux episodes were measured following a meal. As would be expected, the 12 hour overnight studies confirmed that healthy control subjects as well as esophagitis patients had episodes of gastroesophageal (GE) reflux. The patients, however, had a significantly greater number of reflux episodes per 12 hour interval than the controls (30.16 ± 16 SD vs 10 ± 8 SD, p less than .05). For both groups reflux frequency increased after eating (5 per hour vs 2.5 per hour) suggesting that gastric volume plays a role in promoting reflux. The patients were not homogeneous with a great deal of variability being present. The reflux rate tapered off dramatically over 11 hours dropping to a frequency of about 1.4 per hour in patients and 0.2 per hour in control subjects.

Assessment of LESP revealed that considerable temporal variation occurred from minute-to-minute and also between 10 minute or one hour intervals. The standard deviation for the minute-to-minute LESP variation was 6 mm Hg. A representative resting LESP value was obtained for each subject by determining an average value for the 12 hour recording period. Mean 12-hour LESP for the patients, 16 + 7 SD mm Hg, was significantly less than the control subject, 32 ± 12 SD mm Hg (p less than .05). Variability was dramatic. In one normal volunteer LESP was shown to vary between 60 and 20 mm Hg over the one hour period immediately following the evening meal. The amplitude of the variation appeared to diminish over time although this point was not specifically tested.

2. Advances in Gastric Research Investigators at the Alton Ochsner Medical Foundation in New Orleans (Bowen, AM 19897) have been examining the mechanism of injury to canine gastric mucosa during septic shock. The result of IV infusion of live E. Coli organims is a hyperdynamic state, congruous with clinical sepsis and characterized by normal to increased cardiac output and total gastric blood flow, decreased total peripheral resistance and hypotension. Sepsis caused a significant and persistent decline in gastric epithelial pO_2 and membrane potential difference. The rate of fall was dose-related and not related to total gastric blood flow.

Following these observations, the investigators addressed the possibility that peripheral opiate receptors mediate the effects of sepsis on the gastric mucosa. They found that, using a canine model, the hypoxia of the superficial epithelium induced by a systemic infusion of live E. Coli organisms was mimicked by an intra-arterial infusion of naturally occurring opiate B-endorphin. Naloxone reversed the gastric epithelial hypoxia caused by sepsis and also prevented the development of systemic acidosis. The mean blood pH of septic dogs declined during the experiment from 7.42 ± 0.06 to 6.88 ± 0.17, whereas corresponding values for the naloxone treated group were 7.38 ± 0.06 and 7.32 ± 0.08. The salutory effect of naloxone on epithelial oxygenation (with no change in total gastric blood flow) and arterial pH and the similarity of the gastric response to B-endorphin infusion compared with sepsis supports the proposition that local opiate receptors, known to be present in the gastrointestinal tract, may mediate some of the effects of sepsis.

A great deal of attention has been given to the role of prostaglandins (PG) in the macroscopic cytoprotection of the gastric mucosa to noxious agents. Investigators at Harvard University (Ito, AM 26151) have evaluated the microscopic morphology of the rat gastric mucosa exposed to absolute ethanol both with and without PG treatment. Semi-thin sections from areas of the stomach that appeared undamaged by visual inspection and areas of necrotic lesions were examined. Severity of damage to the musoca was graded according to destruction of only luminal surface cells, or luminal and gastric pit cells, or damage extending into the gastric glands. They showed that: 1) PG or 5% ethanol which was the vehicle for the PG caused damage to less that 5% of the gastric mucosal surface and this effect was all superficial. 2) All stomachs receiving absolute ethanol, whether PG treated or not, had about 78% of the gastric surface damaged. 3) The necrotic leison areas were conspicuous red or dark bands which were regions of deep damage with hemorrhage and hyperemia. 4) The formation of these lesions was prevented by PG treatment but the amount of gastric mucosa damaged was still about 75%. However, PG did reduce the amount of the deep damage extending into the gastric glands. The investigators have suggested that the term "cytoprotection" which has been used to describe the action of PG on the prefention of gastric necrotic lesions is an inaccurate and inappropriate term because prostaglandin treatment does not prevent cellular damage when stomachs are exposed to alcohol.

Other grantees at Harvard (Szabo, AM 00600) have been examining the susceptibilityresistance cycle of rats to the ulcerogen, cysteamine. Their results indicated a dramatic difference in the ability of cysteamine to produce duodenal ulcers when given either subcutaneously or orally. When considering both the number of ulcers and the severity of the lesions, it was found that female rats were most susceptible to the ulcerogen when it was administered between 10 and 14 hours after the lights were turned on in the animal room. Mortality in the animals was also greatest in those animals injected at 10 and 14 hours after the lights were on. Gastric acidity was assessed in fasted rats by measuring the titrable acidity 2 hours after injection of cysteamine at each of the injection time points. Acid content was found to be lowest in animals injected 2 and 6 hours after lights on, while acid content peaked in animals injected at 14 hours after lights on. The authors conclude that the effect of cysteamine on duodenal ulceration and gastric acid secretion has diurnal variation, and it is maximal when the ulcerogen is injected at 10:00 or 14:00 hours after lights on.

3. Advances in Large Bowel Research The preponderance of research activity in large bowel supported by this program is with the study of inflammatory bowel diseases. Two general avenues of investigation are the study of (1) immune status and and immunologic dysfunction and (2) cytotoxic agents each of which may be involved to some extent in the inflammatory disease process.

Investigators at Duke University (Roche, AM 27260) have been investigating autoimune mechanisms which might lead to inflammatory bowel disease. Using a rat animal model, this research team is testing the hypothesis that an immune response to a gut organ-specific antigen can be etiologic for gastrointestinal inflammatory leisons seen in experimentally immunized animals. Good progress has been made in developing antibodies to purified intestinal components of animal mucosa and in developing sensitive methods for detecting the autoimmune responses in animals. The investigators believe that the identification, isolation and immunologic characterization of immunogenic organ-specific components associated with bowel epithelial cells (the putative target in ulcerative colitis), may lead to the study of similar potentially disease-inducing components in man.

Carrageenan, a mucillagenous derivative of dried red algae, has been used to produce colitis in guinea pigs. Grantees at Tufts University (Onderdonk, AM 26453) have been investigating the relationship of different bacterial species to the production of carrageenan colitis. They have found, for example, that obligate anaerobes were essential for the development of the lesions, but that pretreatment with metronidazole or clindamycin prevented the lesions. Delaying treatment until the ulcers developed eliminated the therapeutic effect of these two compounds. Evidence suggested that carrageenan, per se, was not responsible for for inducing the colitis. Using gnotobiotic or germ-free animals they attempted to determine what bacterial species might be involved in the colitis process. The agent was Bacteriodes vulgatus. This bacterial strain was capable of producing cecal ulcerations in the presence or absence of carrageenan treatment.

Additional characterization of cecal content from animals with ulcerative leisons and stool from patients with acute, active ulcerative colitis or other inflammatory bowel diseases revealed three factors of interest. These factors were (1) increased chemotactic activity, (2) a cytophatic effect on an in vitro cell culture line (Vero cells) cells, and (3) the presence of certain unsaturated fatty acids (LCFA), particularly C18:2 and C18:3. These factors were not found in cecal content from normal animals and non-ulcerative colitis stool samples. The lipid soluble chemotactic factor found in stool appeared to be the unsaturated

C18 fatty acids. Studies to determine what role prostaglandins might play in this process are underway. The investigators are also seeking to identify substances in the feces which are cytotoxic for tissue cultures of human and other cell lines.

4. Meetings Two meetings of advisors were held by program are 5A in the past ten months.

On September 18, 1981 approximately 30 investigators met at the University of Minnesota at the conclusion of the 15th biennial meeting of the International Society of Chronobiology to discuss the role of chronobiology* in the study of the digestive system. The meeting was organized and sponsored by NIADDK's Digestive Diseases and Nutrition Program (DDN) with the assistance of the University of Minnesota. Attendees included many international authorities on chronobiology who have worked totally or partially on the study of gastrointestinal function and pathology.

Time dependent variability has been shown to be an important parameter to be considered in experimental design and the interpretation of scientific data. Statistically significant time-dependent variability has been found in such parameters as body temperature, blood pressure, red blood cell count, the ability of a organism to resist noxious agents such as oncostatic drugs, and many other physiological and pathophystiological processes. Such proven variability seriously challenges the long held notion of homeostasis which suggests that an organism's internal conditions are invariant with respect to changing environmental conditions and that physiologic, time-dependent changes in body functions are insignificant and without importance.

The purpose of the conference was to examine the kinds of work which has already been done on periodic changes in the digestive system and to suggest areas of needed research in the digestive system in which the time factor must be considered.

A wide variety of observations was presented. Some of these were:

1. Liver cell injury and hepatic drug metabolism are two areas where substantial literature already exists demonstrating the existence of naturally occurring rhythms.

2. Absorption appears to be affected by naturally occurring rhythms. Such a changing state of conditions would profoundly affect the nutrient value of various foodstuffs ingested at various times of day.

3. Cell division in the gut is highly rhythmical on a circadian basis and reproducibly so. Consequently, experiments dealing with cell kinetics in the gut must take that naturally occurring variation into account.

4. Epidermal growth factor, insulin, glucagon, and ACTH all exert circadian effects and are secreted in a circadian fashion.

5. There is a circadian-stage-dependent relationship of pentagastrin on DNA synthesis in mouse esophagus, stomach, colon, and rectum.

One of the recommendations of the committee was that experienced gastrointestinal investigators should be brought together with chronobiologists to study GI function.

On January 21-23 a small group of scientists from varying disciplines convened at NIH to address the possible role of fiber-optic endoscopy in digestive diseases research. The meeting was organized and sponsored by DDN with the assistance of the Executive Committee of the American Society for Gastrointestinal Endoscopy (ASGE). The meeting was attended by basic scientists, engineers, clinicians and manufacturers of endoscopic devices and their associated equipment.

Fiber-optic technology in the form of endoscopic devices has made it possible to examine the interior of the gastrointestinal tract, take biopsies and remove polyps. Endoscopy has led to major changes in the clinical practice of gastroenterology. For example, endoscopy has made it possible to identify the sites of upper gastrointestinal bleeding by direct observation and has shown that erosions and not ulcers are the common cause of hemorrhage. Endoscopy has also made it possible to remove polyps without abdominal surgery. The purpose of this conference was to examine how this technology could be used in research on the normal functions and diseases and disorders of the digestive tract.

In order to focus the discussions, deliberations were confined to three specific areas, each chaired by a different scientist: Circulation, Dr. Eugene Jacobson, University of Cincinnati; Morphology, Dr. Erhard Haus, St. Paul-Ramsey Medical Center, Minneapolis; Secretion, Dr. John Walsh, UCLA. After initial background presentations which covered each area and described new instruments and instrument capabilities, the subgroups discussed their respective areas of concern in simultaneous sessions. Each subgroup then reported back to the full committee with specific recommendations for uses of endoscopy in research. Some of the recommendations follow:

° Development of a quantifiable method for the continuous measurement of mucosal blood flow is needed; such observations could have relevance to the development and healing of stress ulcer and peptic ulcer and help distinguish betwen ischemic colitis, ulcerative colitis, antibiotic colitis and Crohn's disease of the colon; the laser-doppler velocimeter was identified as being one method which might be senstitive to rapid changes, provide continuous monitoring, and produce quantifiable results.

° A two channel endoscope fitted with a hydrogen electrode (to measure resting blood flow) and a laser-doppler velocimeter (to measure changes in blood flow) together could provide a very sensitive record of blood flow.

° Technology now exists to use a magnifying endoscope to visualize the gastric mucosa microcirculatory pattern using either epi-illumination of fluorescein in the vasculature or trans-illumination in patients who would be undergoing peritoneoscopy or laporoscopy. These techniques could be used to visualize areas of

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*Chronobiology is defined as the science of objectively quantifying and investigating mechanisms of biologic time structure, including the rhythmic manifestations of life.

healed or active ulcers. There are other techniques which may be used but the biggest difficulty is that whatever technique is developed, it must produce numbers related to mucosal blood flow.

° It may be possible to measure the tension in the wall of a varix and predict the possibility of rupture.

° Studies are required to understand the behavior and function of the normal epithelial tissue of the GI tract including the chronobiology of cell kinetics and proliferation and the effect on these functions of exogenous agents including carcinogens. Such studies could contribute to our understanding of the healing of gastrointestinal leisons.

° Endoscopy offers the opportunity for longitudinal observations and biopsies in those patients with leisons or disease states, such as atrophic gastritis, which are associated with increased cancer risk.

° Instrumentation is needed for the immediate freezing of biopsy tissue so that histochemistry and morphology can be studied before the deterioration that occurs so rapidly in excised, digestive tract tissue.

° It would be useful to develop an endoscopically applied collecting cup for collecting a mixture of pure pancreatic and biliary secretions, uncontaminated by intestinal contents. Such experiments may contribute to an understanding of the cause and a treatment for pancreatitis.

° Intraductal probes can be used to measure pH, CO_2, Ca^{+2}, etc. continuously at one site or at various levels of the duct. Thus, changes in the secretions, as they pass through the ductular system, could be determined.

° In the stomach, topical physiological probes are needed to identify areas of abnormal secretory function and relate these to markers of tissue metaplasia.

° Because of the ability of clinicians to catheterize the biliary ductal system, new solvents and methods for delivering these slovents for the direct dissolution of gallstones, common duct stones and retained stones can be studied.

° Because of the development of miniature fiberoptics, it is possible to implant a fiber bundle in the GI tract of animals and exteriorize the distal portion of the bundle percutaneously. This would allow visualization of the region under study at any time and following any treatment with a minimum of disturbance to the experimental animal. A device to wash the end of such a bundle may need to be developed.

° The possibility was raised that under selected circumstances, it may be possible to conduct spectrophotometric measurements through a fiber bundle in an endoscope and thus monitor chemical changes.

LIVER and BILIARY TRACT DISEASES PROGRAM

Sarah C. Kalser, Ph.D., Program Director

The program supports studies of the structure, function and diseases of the liver and biliary tract. Disease entities include inflammatory, metabolic and genetic diseases of the liver such as hepatitis, cirrhosis, Wilson's disease, primary biliary cirrhosis, fatty liver, hepatic encephalopathy, Dubin-Johnson syndrome and Gilbert's disease. It also includes studies on liver regeneration, liver assist and liver transplantation as well as liver ischemia, portal hypertension and toxic liver disorders. The biliary tract and its diseases include cholestasis, cholesterol and pigment gallstones, and bile salt, bile pigment and cholesterol matabolism.

The Liver and Biliary Diseases Program utilizes many funding mechanisms to implement the program including regular research grants, program projects and centers, career development awards, clinical investigator awards, new investigator research awards, training grants, fellowship and contracts.

Increased interest and research findings about liver transplantation brought about a planning meeting (June 8, 1982) to result in a Consensus Conference in 1983.

I. Research and Center Grant Activity

1. Relationship of Hepatitis B virus integration and Chronic Active Hepatitis
There is now evidence, at the molecular level, that the hepatitis B viral genome (or parts of the genome) is not integrated into the DNA of the liver cell in chronic active hepatitis carriers of less than two years. It is integrated into the DNA in carriers of more than 8 years and in hepatocellular carcinoma (D. Shafritz).

In both a chimpanzee animal model and people, it is now apparent that years after infection with hepatitis B virus (HBV), a chronic carrier state leading to chronic active hepatitis (CAH) can develop. Chimpanzee carriers some 10-14 years after infection are now showing CAH. They are also showing viral DNA in the liver cell nucleus but no integration of this viral DNA into the liver cell DNA. In contrast, in a human hepatocellular carcinoma cell line in tissue culture, HBV DNA has recently been found to be integrated into the host genome. With development of newer techniques in nucleic acid analysis and the availability of recombinant cloned HBV DNA probes, this question can be addressed in humans using small amounts of tissue obtained from liver biopsy samples.

The findings are that in human liver biopsy specimens from chronic active hepatitis HBV-carriers, viral DNA, but no integration into the host genome was found in five carriers of hepatitis B surface antigen (HBs Ag) who had evidence of the carrier state and liver disease for less than 2 years. In the two patients who had carried HBs Ag for more than 8 years, HBV-DNA was integrated into the host genome. In human primary hepatocellular carcinoma, the DNA extracts from all tumors coming from patients who were HBV carriers showed HBV-DNA integrated into the host genome.

This has medical significance. There are approximately 180 million HBV carriers worldwide (750,000 in the U.S.). Epidemiologic and other evidence suggest an etiologic relationship between HBV infection and primary hepatocellular carcinoma. Because chronic active hepatitis may be a sequelae of the carrier state in 1-5%, and there is a current controversy about the treatment of CAH, it becomes important to learn whether unresolved CAH becomes primary hepatocellular carcinoma. Future research which sequentially follows all stages in HBV infection from onset of acute hepatitis to chronic liver disease and maybe even to carcinoma is now feasible. This may give leads as to when therapeutic intervention could be most meaningful.

2. H_1+ H_2 - Histamine Receptors in the Gallbladder Histamine receptors have been investigated in many organ systems, but it is only after the development of specific H_2-antagonists that the potentially complex role of histamine in the regulation of smooth muscle activity has been appreciated.

A long-time NIADDK grantee in Boston (Lester F. Williams, Jr.) has demonstrated for the first time the presence of both H_1 and H_2 histamine receptors in a primate species. Even though contraction is the only response evoked from the gallbladder when histamine is administered, this grantee has been able to dissect the system using an H_1-specific agonist (2-pyridylethyamine or 2 PEA) and an H_2-specific agonist (2- (3- (N, N-dimethylamino) propyl isothiourea) or Dimaprit. They also used specific antagonists or histamine-blocking drugs; benadryl to block the H_1-receptor and metiamide to block the H_2-receptor.

Dr. Williams and colleagues showed that in unsedated baboons, the H_1-histamine receptor caused gallbladder contraction whereas the H_2-histamine receptor caused gallbladder relaxation. Although a similar relationship for H_1 - and H_2-receptors has been described in other smooth muscle containing organs, the physiologic significance of this finding with respect to gallbladder contraction is unclear. The role of histamine in controlling the contractile state of the gallbladder is as yet undefined but these studies do raise the possibility that the H_1-receptors facilitate gallbladder emptying during a meal and that H_2-receptors aid in refilling after a meal.

3. Controlled Trial on Surgical Procedures for Cirrhotic Patients with Esophagogastric Hemorrhage Patients with cirrhosis often have such contriction of the portal venous system that portal hypertension occurs. The body circumvents this obstruction in liver blood flow by developing collateral veins, which may go to the esophagus, stomach or rectum. The primary consequences of portal hypertension are ascites, hemorrhage from esophagogastric varices and portal-systemic encephalopathy (PSE). Hemorrhage from the varices is the most lethal of the complications of portal hypertension; about 50% of patients with this disorder die. The treatment of bleeding varices ranges from esophageal tamponade, to obliterative methods such as endoscopic sclerosis, to surgical decompression of the portal hypertension.

Surgical treatment in which blood from the portal vein is shunted around the liver into the main vein of the body, the vena cava, has been the treatment of choice for patients who have bled from esophageal varices (portal systemic shunt or PSS). Unfortunately, even though future bleeding is controlled, the operation often leads to severe and recurrent encephalopathy and also, an accelerated mortality (not due to bleeding) in patients 2-5 years after creation of the shunt. In an attempt to avoid these post-shunt syndromes, Warren et al (NIADDK grantees) devised in 1967 a two-phase operation which decompresses the varices and allowed portal blood to perfuse the liver. This later operation, the distal splenorenal shunt (DSRS) is more technically difficult but was thought in early studies to show lower incidence of encephalopathy than the PSS.

This report concerns the latest in a series of clinical trials and was performed at 6 cooperating hospitals in the Boston - New Haven area. The operations were performed by 12 different surgeons and was coordinated by two NIADDK grantees (H.O. Conn and R.H. Resnick). It was initiated in 1975. The goals of the study were to compare, for the two types of portal decompressive surgery, the following criteria: operative and late mortality, prevention of subsequent hemorrhage from varices, the frequency and severity of encephalopathy, and hepatic failure. Interim results were reported in 1981 and are presented here, although the trial is still ongoing to obtain even more long-term results. Unlike reports from other studies, this group failed to find any significant reduction in encephalopathy following DSRS. All other parameters evaluated were also similar for both groups: in hospital and late mortality, frequency of rebleeding, technical failures to construct the shunt, and subsequent malfunction of the shunt. Assessing all these aspects cumulatively, the two shunt operations appear equally efficacious and equally hazardous. Fewer than 60% in the 2 groups were free of lethal or serious problems associated with the procedure. Another interesting observation is that a small group of patients who were eligible for the study but refused surgery had the same long term survival rate as did those having surgery and they did not have episodes of encephalopathy, compared with about 21% in the combined surgically-treated groups. They did, however, show a higher incidence of hemorrhage from variceal bleeding.

4. A new form of cholesterol is found in adult human serum. Dr. Norman Javitt (Cornell) was able to quantitate free and esterified 26-hydroxycholesterol (cholest-5-ene-3B-26-diol) in adult serum using mass spectrometry and an isotope dilution method. This is significant because it was thought that this major sterol of human meconium is not found after neonatal life. Dr. Fredrickson, in 1956, showed that mouse liver mitochondria could metabolize cholesterol to both the -25 and -26 hydroxycholesterol, of which the -25 turned out to be auto-oxidation product or artifact.

A very significant preliminary finding is that persons with cerebrotendinous xanthomatosis (CTX) have no 26-hydroxycholesterol in their serum whereas the normal level is 9-25 ug/100 ml (free sterol 31-35%). If these preliminary findings are verified with larger numbers, it could provide a significant diagnostic test in many cases where neurologic dysfunctions may be misdiagnosed as depression, brain damage, etc., whereas they are really victims of early CTX. The full-blown disease with tuberous and tendon xanthomas, premature atherosclerosis, pulmonary insufficiency, cataracts and endocrine malfunction can be diagnosed but early neurologic signs may be missed. The disease is one in which virtually every tissue accumulates cholesterol and cholestanol due to abnormal cholesterol metabolism.

This NIADDK investigator postulates that 26-hydroxycholesterol could be a key regulator of cholesterol synthesis, reducing it by inhibiting 3-HMG CoA reductase as has been shown in liver cell cultures. When this metabolite (26-hydroxycholesterol) is low or missing, cholesterol synthesis goes unchecked and cholesterol deposits build up in the various organs involved in CTX.

Other grantees (G. Salen and E. Mossbach) think the defect in CTX is due to defective bile acid synthesis which forces more cholesterol to be produced to provide substrate for making bile acids. They have administered the bile acid, chenodiol, to subjects to reduce the cholesterol and cholestanol overproduction.

5. Maturation of Liver Function is suppressed by an intrauterine milieu Many naturally occurring compounds such as steroids and bilirubin as well as exogenous xenobiotics such as chloramphenicol and aminobenzoate are cleared by the liver by conjugation with glucuronic acid and excretion into the bile or urine. This process, which makes the original compound more water-soluble and less biologically active, is catalyzed by an hepatic microsomal enzyme bilirubin UDP-glucuronyl transferase (BGt).

This enzyme (BGt) is normally absent or of very low activity at birth but increases rapidly after delivery so that it achieves adult levels in the first 4 days of neonatal life. To test whether it is the age of the liver or the internal environment that allows maturation and rapid increase of the enzyme activity, this NIADDK investigator (G. Odell) produced an experimental model in which the fetuses of rats were forced to have a 23-24 day gestation instead of the normal 21 day gestation. When these postmature rats were delivered, the level of BGt was equal only to that of the just-delivered normal gestation time rat (21 days). In other words, they did not behave like would be expected for their age which was equal to 2-3 days post-gestation. On electron microscopy, the liver of the post-mature rat also looked like the immature liver - very little smooth endoplasmic reticulum.

These data are consistent with the hypothesis that BGt activity of fetal liver in utero remains suppressed for at least two reasons: (1) the fetal liver is exposed to high levels of circulating gestational hormones, particularly estrogen which is known to inhibit bilirubin clearance, and (2) the placenta efficiently clears bilirubin formed by the fetus in utero so that the circulating concentrations of this potential inducer remain low.

These findings are interpreted to suggest that the intrauterine milieu actually suppresses fetal maturation of liver function.

II. The two Liver Centers, University of California at San Francisco and Albert Einstein Medical College, have utilized some of the funds provided their respective Centers to support pilot or feasibility projects. These are usually innovative research ideas proposed by young investigators within the Center which may be high risk, high gain ventures. The funds may also be used to obtain enough preliminary data to test the feasibility of a full scale project. The projects are written up formally, and reviewed by a group of outside advisors to the Liver Center. Internal guidelines suggest that the project be only 1 year in duration (maximum of two years), not a supplement to ongoing funded work, and be of modest cost (usually $15,000 - $25,000).

Some of the projects supported as pilot/feasibility projects during the past year were:

1. University of California, San Francisco:

   a. Development of a method for in vitro perfusion of a wedge biopsy of human liver to be used in morphological and functional studies of the liver.

b. The drug-induced displacement of unconjugated bilirubin from plasma to bile; the finding of bile pigments in the bile of the electric torpedo fish.

c. Development of the technique to measure membrane potential, membrane resistance and cation fluxes using microelectrodes in cultured rat hepatocytes.

d. The finding of an interesting animal model, the blotchy mouse, which has an unusual pattern of copper accumulation and secretion and which may compare to the human disease, Menkes' kinky hair syndrome.

2. Albert Einstein College of Medicine

a. The attempt to infuse hepatocytes into the portal vein to correct metabolic diseases in mutant animals.

b. An initial study to prepare vesicles from plasma membranes of rat hepatocytes so that organic anion transport can be studied.

c. The attempt to use lipoprotein receptors to target metabolites, drugs and chemicals to the liver.

d. The use of nuclear magnetic resonance to study glycolysis and gluconeogenesis in regenerating rat liver.

e. In collaboration with Prof. Kew, South Africa, the finding of hepatitis B viral DNA in liver cancer and chronic hepatitis.

III. Contracts
National Cooperative Gallstone Study (NCGS)
N01-AM-0-02205 FY 81 = $223,094

Of the 916 patients with cholesterol gallstones who were treated with either high dose, low dose chenodeoxycholic acid or placebo for a two year period in order to dissolve their gallstones, 53 out of 59 eligible patients enrolled in a Phase III study. This study is a follow-up to the dissolution study with the aim of determining whether recurrence of gallstones will occur when treatment stops, and whether low dose chenodeoxycholic acid administration will prevent recurrence. These patients are being followed in this randomized trial for 2 years. Patient treatment will end August 1982 and the data will be analyzed.

INTESTINAL AND PANCREATIC DISEASES PROGRAM

Gerassimos G. Roussos, Ph.D.

In FY 81, 96 applications (AM primary assignments) were assigned to the Program. Sixty percent (60%) or 52 of the 86 applications approved were funded. As of March 31, 1982, approximately 66% (99 activities), 20% (30 activities), 10% (15 activities), and 4% (6 activities) of the 152 activities under support were within the small intestine, pancreas, gastrointestinal hormone, and salivary gland areas, respectively, constituting only a slight change in overall subject area distribution since last year's annual report. This change was reflected by a minor increase in the small intestine area and a small decrease in the pancreas area, in spite of efforts to stimulate the latter area via the support of a workshop and the release of a program announcement in this connection. During the past year, there has also occurred a 57% decrease in postdoctoral fellowships and a 133% increase in new investigator research awards under support by the Program due primarily to a significant decrease in the training and "research career award and other reasearch" budgets, respectively. Increase in available funds will obviously help reverse at least some of the declines in the number of activities under support noted above.

Although a considerable amount of research and other activities, both basic and clinical, has been either reported by the grantees or initiated by the Program during the past year, only a few examples will be cited to illustrate the progress that has been made in various areas of interest to the Program.

1. Small Intestine: (a) Microtubules are ubiquitous cytoplasmic proteins which have been implicated in cell motility, mitosis, and a variety of secretory processes, including those for neurotransmitters, hormones, and enzymes. They are elongated helical polymers of the protein dimer, tubulin, which consists of two 55-60,000 mol wt monomers. A dynamic equilibrium between tubulin and its polymers (microtubules) is maintained by various cytosolic factors, including guanine nucleotides, Ca and Mg. With respect to their role in secretory processes, microtubules appear to facilitate the intracellular movement of granules or vesicles to the plasma membrane. These then fuse with the plasma membrane resulting in (a) extrusion of their contents from the cell and (b) an alteration in the surface area and composition of the plasma membrane with a resulting change in permeability properties. The ability of certain drugs, such as colchicine (but not its structural isomer, lumicolchicine) and vinblastine, to bind to tubulin and thereby prevent its polymerization has been extensively used to explore the functions of microtubules. In addition to a large body of literature showing inhibition by colchicine and vinblastine of neurotransmitter, hormone and protein secretions, there are also a few reports on inhibitory effects of these drugs on water and electrolyte secretion (salt excretion by marine teleost gills, acid secretion by gastric mucosa and turtle urinary bladder, and cholera toxin-induced fluid secretion in rat small intestine). The antisecretory effect in the intestine was only demonstrated in vivo, and it could not therefore be established whether colchicine affected the epithelium directly or only indirectly through neural, hormonal, or vascular effects. It was also not established whether colchicine inhibited cholera toxininduced accumulation of cyclic 3',5'-AMP (cAMP) or whether it inhibited a step in the secretory process beyond cAMP accumulation.

Dr. Field et al. (R01 AM 21345) at the University of Chicago, Harvard School of Medicine, and Beth Israel Hospital, Boston, Massachusetts have recently attempted to further define how inhibitors of microtubule assembly inhibit intestinal secretion by determining the effects of colchicine on cholera toxin and prostaglandin-stimulated fluid production and adenylate cyclase activity in vivo and also the effects of colchicine, lumicolchicine and vinblastine on cAMP and carbamylcholine-stimulated secretion in vitro. In the intact rat, intraperitoneal injection of colchicine (5 mg/kg) inhibited cholera toxin and prostaglandin-stimulated secretion without inhibiting their effects on adenylate cyclase or cAMP concentration. Pretreatment with colchicine had no effect on fluid transport in the absence of secretory stimuli. When added to rat ileum in vitro, colchicine reduced by 60% the short-circuit current (I_{SC}) response to dibutyryl cAMP added 4 h later, whereas its structural isomer, lumicolchicine, which does not inhibit microtubule assembly, was ineffective. Vinblastine reduced by 55% the I_{SC} response to dibutyryl cAMP and theophylline added 2 h later. Two hour pretreatment with vinblastine also reduced by 40% the I_{SC} response to the cholinergic agonist, carbamylcholine, a Ca-dependent secretory stimulus which does not increase cAMP concentration. In contrast to their antisecretory actions, neither colchicine nor vinblastine inhibited glucose-stimulated active Na absorption. These results suggest a role for microtubules in active electrolyte secretion in the small intestine.

(b) Since the early 1960s the "Na^+-gradient hypothesis" has emerged as a plausible model for Na^+-dependent active transport processes in the intestine. The model holds that Na^+ and an organic solute are transferred together across the brush-border membrane by a carrier system which allows coupled facilitated diffusion of both substrates. The movement of Na^+ from the lumen into the cell down its electrochemical potential gradient provides the energy for the uphill transfer of the solute across the brush-border membrane. In order for the cell to maintain a low intracellular Na^+ concentration necessary for continued operation of the system, this cation is pumped out of the cell with energy derived from adenosine triphosphate (ATP) hydrolysis by sodium-potassium-activated adenosine triphosphatase (Na^+ K^+-ATPase). The transport system in the ileum for bile acids satisfies many of the criteria for active transport. That the $Na^=$-gradient hypothesis applies to active bile acid absorption is supported by the findings that the ileal transport is: (a) Na^+-dependent in whole tissue segments, (b) sensitive to ouabain in isolated cells, and (c) the result of a secondary active Na^+-dependent transport involving the coupling of Na^+ and bile acid flux across brush-border membrane vesicles. Inherent in the Na^+-gradient hypothesis is that bile acid transport should be dependent upon the activity of Na^+, K^+-ATPase. That the activity of Na^+, K^+ -ATPase may control the rate of intestinal absorption is suggested by the finding that the increase in sodium and water absorption in rat ileum produced by glucocorticoids was associated with increased activity of Na^+, K^+ -ATPase. However, reports on the effect of ouabain, a known specific inhibitor of Na^+, K^+ -ATPase, have not shown consistently a correlation between electrolyte transport and Na^+, K^+-ATPase activity in the intestine. Also inherent in the model system is that bile acid transport is dependent upon the Na^+ gradient across the intact mucosal cell. It follows that an agent, such as gramicidin D, which increases membrane permeability to cations, should affect bile acid transport.

Studies were recently performed by Dr. Wilson et al. (R01 AM 21696) at Vanderbilt University School of Medicine to determine relationships among Na^+, K^+-ATPase, the transmucosal Na^+ gradient, and bile acid transport in metabolically viable cells isolated from rat ileum. Incubation of cells with 0, 10^{-6}, 10^{-5}, 10^{-4} and 10^{-3} M ouabain resulted, respectively, in a 0, 10.3, 42.1, 97.0, and 100% decrease in glycocholate uptake and a 0, 10.7, 46.4, 76.8, and 100% decrease in Na^+, K^+-ATPase activity. Thus, one-half maximal inhibition of glycocholate uptake and Na^+, K^+-ATPase activity occurred at 5.5×10^{-5} M and 1.7×10^{-5} M ouabain, respectively. A change in glycocholate uptake was correlated with a change in NA^+, K^+-ATPase activity after daily injections of methylprednisolone. After 4 days, treated animals showed a 26% and 36% increase in glycocholate uptake and Na^+, K^+-ATPase activity, respectively, over pair-fed saline-treated controls. Methylprednisolone did not significantly alter the activity of (Mg^{++})-ATPase when compared with controls. Glycocholate uptake was reduced by the omission of Na^+ from the incubation medium. Preincubation of cells at 37°C with gramicidin D, 10 ug/ml, to alter membrane permeability to Na^+ resulted in a significant rise in cell $Na^+$and a significant fall in glycocholate uptake from values in untreated cells to approach values for glycocholate uptake at 0°C. These data suggest that Na^+, K^+-ATPase may play a role in bile acid uptake into ileal cells possibly by maintaining a Na^+ electrochemical potential gradient for coupled Na^+-bile acid transport.

(c) Somatostatin (SRIF) and the enkephalins are peptides recently found to be present in the intestine. SRIF is 14 amino acids in length and the enkephalins are composed of 5 amino acids. The two naturally occurring enkephalins differ only in their terminal amino acid, one being methionine, the other being leucine. SRIF and the enkephalins are present in both mucosal endocrine cells and neurons in the intestine. Therefore, they may function as neurotransmitters or have a paracrine effect in the intestine. In addition, SRIF may function as a circulating hormone. Both SRIF and the enkephalins have been shown to affect a variety of intestinal functions. SRIF inhibits gastric acid secretion, pancreatic enzyme and bicarbonate secretion, intestinal motility, and splanchnic blood flow. It is a potent inhibitor of enteric hormone release. The enkephalins augment histamine-induced gastric acid secretion, inhibit pancreatic enzyme secretion, and decrease intestinal motility.

Since SRIF and the enkephalins are present in the intestine and affect a variety of intestinal functions, Dr. Binder et al. (R01 AM 14669) at Yale University School of Medicine considered it likely that they also had a direct effect on intestinal electrolyte transport. Studies were carried out to determine the effect on electrolyte transport, the mode of action, and potential therapeutic application of these peptides. SRIF and enkephalin stimulated net Na and Cl absorption in the rabbit ileum and appeared to do so primarily by stimulating the coupled influx of Na and Cl across the brush border membrane. Not only could SRIF stimulate absorption in the rabbit ileum, it could also inhibit the effect of secretagogues in the rat jejunum and rat colon. SRIF infusion alone had no effect on basal water movement in the rat jejunum in vivo. Prostaglandin E_1 (PGE_1) inhibited net water aborption and SRIF infusion completely blocked the inhibition of water absorption by PGE_1. SRIF also blocked the effect of serotonin and theophylline on ion transport in the rat colon. Serotonin decreased net Na and Cl absorption. SRIF completely blocked the effect of serotonin on these parameters. Theophylline abolished net Na absorption and converted net Cl absorption to secretion. SRIF completely reversed the effect of theophylline on Cl secretion, but only partially reversed the effect of theophylline on Na absorption. SRIF had no effect on the increase in cyclic AMP levels induced by theophylline and PGE_1 in isolated colonic epithelial cells. These results indicate that SRIF can block the effect of cyclic AMP-dependent (theophylline and PGE_1) and cyclic AMP-independent (serotonin) secretagogues without affecting cyclic AMP levels. This suggests that SRIF is working at some distal step in the secretory process beyond cyclic AMP formation. The ability of SRIF to reverse or prevent secretagogue-induced secretion in the rat jejunum and ileum suggests that SRIF may be therapeutically useful in secretagogue-induced diarrhea. Dr. Binder et al. have infused SRIF into a patient with the carcinoid syndrome and watery diarrhea. During the SRIF infusion, diarrhea ceased and there was a dramatic decline in stool weight and electrolyte content. These results have been confirmed by another laboratory in another patient with secretory diarrhea due to malignant carcinoid syndrome. An oral analog of SRIF is required before chronic studies of SRIF's usefulness can be determined.

(d) Peyer's patches and the appendix are the major discrete lymphoid organs of the gut. Lymphoid follicles that form Peyer's patches bulge between villi into the intestinal chyme flow. Surfaces of these lymphoid follicles have reduced numbers of goblet cells and, in addition, are made up of cuboidal cells and even shorter M cells, which are more heavily infiltrated with lymphocytes than anywhere over villi. Over Peyer's patch follicles, relatively sparse numbers of goblet cells create a breach in the mucus blanket allowing particles to reach M cells which transport antigenic material from the intestinal lumen into the space between cells where it can be taken up by lymphocytes. Similar antigentrapping cells are found over lymphoid follicles in the appendix and the cecal patch, which is its homologous equivalent in the mouse. In contrast to villus columnar cells, there is an absence of well-developed, closely packed microvilli over M cells, which permits the approach of luminal particles; in addition, there is an absence of a developed terminal web that allows clusters of migrating lymphocytes to press close to the intestinal lumen. The rudimentary microvilli, shortness, and variability in shape of M cells resemble features of proliferating intestinal crypt cells, even though the latter are never infiltrated by lymphocytes. These characteristics of M cells suggested that they also might be proliferating cells, generating the relatively goblet cell-free epithelium over lymphoid follicle surfaces.

Because of the importance of Peyer's patches in initiating immune responses, the role of M cells in antigen uptake from the intestinal lumen, and the structural difference between follicle associated epithelium and the epithelium lining the remainder of the intestinal tract, the process of cell renewal for lymphoid follicle epithelium is of particular interest. By autoradiography in mice Dr. Owen et al. (R01 AM 21969) at the Veterans Administration Medical Center, San Francisco, California found that epithelial renewal over follicles only occurs by proliferation in multiple adjacent crypts with cell migration onto follicle surfaces. Nuclear labeling of M cells was observed 72 h after [^{3}H]thymidine injection among fully differentiated cuboidal cells from which M cells appear to develop. Goblet-cell flow from surrounding crypts supplied large numbers of goblet cells to adjacent villi, but it was remarkably restricted onto the Peyer's patch follicle surfaces, creating a breach in the mucus barrier that may facilitate antigen sampling.

(e) Cobalamin (vitamin B_{12}) is synthesized only by microorganisms, but is required by man and other animals as an essential cofactor for several enzymatic reactions. Cbl (cobalamin) is bound predominantly by R protein in gastric juice and becomes bound to intrinsic factor after the R protein moiety is partially degraded by pancreatic proteases in the jejunum. The intrinsic factor-Cbl complex then binds to receptors that are present on ileal brush borders, but not on jejunal brush borders. These receptors are specific for the intrinsic factor-Cbl complex and do not bind free Cbl, free intrinsic factor, or other protein-Cbl complexes. The binding of intrinsic factor-Cbl to these receptors markedly facilitates the absorption of Cbl, although little is known about the subsequent steps in the absorptive process.

Drs. Seetharam (R01 AM 26638) and Alpers (R01 AM 14038) at Washington University School of Medicine have recently reported the successful purification and partial characterization of the intrinsic factor-Cbl receptor from canine ileal mucosa using a newly developed immunoaffinity chromatography technique. The initial purification steps involved solubilization of ileal mucosal homogenates with Triton X-100, followed by ethanol precipitation and dialysis. The soluble receptor was then saturated with hog intrinsic factor-cobalamin and the receptorhog intrinsic factor-cobalamin complex was adsorbed to anti-hog intrinsic factor-Sepharose. After extensive washing, the receptor was eluted with 5 mM EDTA at pH 5.0, leaving behind the hog intrinsic factor-cobalamin that remained bound to the anti-hog intrinsic factor-Sepharose. Gel filtration on Bio-Gel A-5m with 0.1% Triton X-100 gave two peaks of receptor activity with apparent molecular weights of 7,500,000 and 5,000,000, indicating that the receptor aggregates under these conditions. Polyacrylamide disc gel electrophoresis with 0.1% Triton X-100 gave a single protein band that barely entered 4% gels, did not stain for carbohydrate, and coincided with the presence of intrinsic factor-cobalamin-binding ability. Polyacrylamide gel electrophoresis with sodium dodecyl sulfate gave one major band with an apparent molecular weight of 180,000, which upon reduction with 2-mercaptoethanol gave two bands with apparent molecular weights of 59,000 and 42,000. Amino acid analysis indicated the presence of 55% hydrophobic amino acids, less than 1% amino sugars, and gave a value of 222,000 g of amino acid/mol of intrinsic factor-cobalamin-binding ability. The purified receptor showed specific binding with human, hog, and canine intrinsic factor-cobalamin (K_a = 1 to 4 nM^{-1})but not with free cobalamin, free intrinsic factor, or R protein-cobalamin. Antibodies to the receptor have been raised in rabbits.

f) Gluten-sensitive enteropathy (GSE, Celiac Sprue) is a disease that affects the small intestinal mucosa and results in the malabsorption of most nutrients. The disease in children and adults is associated with the ingestion of dietary gluten. Oral gluten challenge of gluten-sensitive individuals results in a loss of the normal small intestinal villous architecture (villous atrophy), most strikingly in the proximal small bowel (i.e., jejunum). GSE is known to be activated by protein(s) in the gliadin fraction of wheat gluten. Gliadin, obtained by ethanol extraction of wheat gluten, contains at least 40 components, which can be assigned on the basis of electrophoretic mobilities to 4 major groups all rich in the amino acids glutamine and proline. Bernardin et al. purified an -gliadin fraction, that was later called A-gliadin, and which has been well characterized. This fraction has been shown to precipitate the lesion of GSE in susceptible individuals. How gliadin(s) result in epithelial cell damage and villous atrophy is unknown. However, one of the striking features of GSE is the infiltration of the jejunal lamina propria with plasma cells and the epithelial cell layer with lymphocytes. There are reports also of increased mucosal antibody synthesis to a variety of dietary antigens and components of gluten in patients with GSE. Finally, the major histocompatibility complex (MHC) alleles HLA-B8,

- w3, and -DRw3 occur at high frequency in patients with GSE. The HLA-D region is now recognized to be analogous to the I-region of the murine H-2 (MHC), which is well established to be involved in immunoregulation. Although such indirect evidence may support an immunologic mechanism as important in the pathogenesis of GSE, little is known regarding the nature, regulation, and specificity of the immune response to the gliadin proteins that cause disease.

Dr. Kagnoff et al. (RO1 AM 17276) at the University of California at San Diego have recently examined the genetic control of the murine humoral immune response to purified A-gliadin. Studies with congenic and noncongenic inbred strains of mice, using a radioimmunoassay and a plaque-forming cell assay developed in their laboratory, revealed that the T-dependent humoral antibody response to A-gliadin is under the control of genes mapping to the mouse MHC. These findings in mice establish a clear linkage between the MHC and the regulation of the immune response to a protein capable of causing human disease, suggesting that human MHC-coded immune response genes may control T-dependent humoral antibody and T cell-mediated immune responses to dietary gliadin in man.

(g) An international workshop, organized by Drs. F. P. Brooks and E. C. Boedeker under the auspices of the American Gastroenterological Association and dealing with the attachment of microorganisms to the intertinal mucosal surface and the relevance of this attachment to the pathogenesis of and therapy for a number of highly prevalent enteric infectious diseases, was held at the Sheraton International Conference Center in Reston, Virginia, on October 9 to 11, 1981. The workshop, supported jointly by this Institute, the National Institute of Allergy and Infectious Diseases, and the Fogarty International Center, included sessions on: (1) enteric bacterial adherence in animal systems; (2) enteric bacterial adherence in human disease; (3) pili; (4) morphology and adherence; (5) intestinal mucus and adherence; (6) intestinal membrane structure; (7) intestinal membrane receptors for bacteria; and (8) bacterial adherence and the immune response. The proceedings of the meeting will be published in the form of a volume of the CRC Uniscience series. In addition, a brief executive summary of the workshop will be published in Gastroenterology.

2. Pancreas: a) Digestive enzymes exported from the pancreatic acinar cell are synthesized on ribosomes attached to the rough endoplasmic reticulum, packaged in the Golgi complex and they then migrate in zymogen granules to the luminal plasma membrane. While it is generally agreed that the final step allowing exocytosis of the granule contents is the fusion of zymogen granule membranes with the acinar cell plasma membrane, the mechanism responsible for this process remains unclear. It has been suggested that membrane protein carboxymethylation, catalysed by the enzyme protein carboxymethylase (S-adenosyl-L-methionine: protein-O-methyltransferase), results in a reduction of negative charges which normally prevent membrane fusion and that this event may be of importance in the process of cell secretion. Protein carboxymethylase has been detected in prokaryotic as well as eukaryotic cells and a role for protein carboxymethylase in secretion from the parotid gland, posterior pituitary gland, and adrenal medulla has been suggested. It has also been detected in the pancreas, but its physiological role in this gland has not been characterized.

Young female mice, fed a choline-deficient ethionine-supplemented diet, die with acute hemmorrhagic pancreatitis. The earliest biochemical change noted in the pancreas of mice fed with this diet is a rise in digestive enzyme content and, morphologically, an accumulation of zymogen granule within acinar cells has been observed. This rise in digestive enzyme content results from impaired digestive enzyme discharge. Mice fed ethionine with a choline-supplemented diet also have impaired enzyme discharge and an increased pancreatic content of digestive enzymes but the changes in enzyme content and discharge are less pronounced than those noted after ingestion of the choline-deficient ethionine-supplemented diet.

Dr. Steer et al. (R01 AM 20302) at Beth Israel Hospital and Harvard Medical School, Boston, Massachusetts have recently partially characterized pancreatic protein carboxymethylase and reported the results of studies dealing with the effect of ethionine administration with and without choline deficiency on the activity of this enzyme. They reasoned that, if protein carboxymethylase does play a role in acinar cell secretion, ethionine could cause impaired enzyme discharge by interfering with protein carboxymethylase activity. They found that protein carboxymethylase of mouse pancreas is both soluble (70%) and particulate (30%). The K_m for S-adenosylmethionine is $7.5 \cdot 10^{-7}$ M and the K_i for S-adenosylethionine is $1.3 \cdot 10^{-5}$ M. Administration of an ethionine containing diet results in a decrease in protein carboxymethylase activity. Ethionine ingestion also increases pancreatic amylase content by interfering with digestive enzyme discharge. The reciprocal changes in amylase content and protein carboxymethylase activity can be detected within 12 h of commencing the ethionine administration and are enhanced by simultaneous choline deficiency. These studies support the hypothesis that protein carboxymethylase plays an important role in secretion of exportable material. Inhibition of pancreatic protein carboxymethylase activity in vivo may be one important mechanism by which ethionine interferes with digestive enzyme discharge.

(b) Acute hemorrhagic pancreatitis with fat necrosis (AHPN) is a severe form of human disease which is thought to result from autodigestion of the organ due to intraparenchymal activation of zymogens and formation of active proteinases. Dr. Lombardi et al. (R01 AM 20706) at the University of Pittsburgh School of Medicine have recently developed an experimental model of AHPN in mice having clinical and anatomopathologic features that mimic closely those of the human disease. The onset of the experimental pancreatitis was shown to follow an intraparenchymal activation of zymogens, and indirect evidence was obtained that the latter may be triggered by cathepsin B_1, a lysosomal proteinase. They have now used this model to investigate whether administration to the animals of various proteinase inhibitors would affect the onset of the disease. AHPN was induced in female mice fed a choline-deficient diet containing 0.5% DL-ethionine. The effect of the various proteinase inhibitors on the induction of the pancreatitis was evaluated using three parameters, the mortality of the animals, the appearance before death of a shock-like state, and the severity of the pancreatic pathology. Treatment of the animals with leupeptin, pepstatin, chymostatin and/or antipain, proteinase inhibitors of microbial origin, resulted in a distinct attenuation of the severity of the induced process, whereas Aprotinin (a bovine trypsin inhibitor) and chloroquine (a reputed inhibitor of cathepsin B_1 in tissue culture systems) had no effect.

Leupeptin, pepstatin, chymostatin and antipain are known to have a broad spectrum of activity and to inhibit not only serine proteinases, such as those of pancreas, but also thiol proteinases, such as lysosomal cathepsin B_1. When the inhibitors were administered during induction of the pancreatitis, they were found to influence all relevant parameters studied. They reduced the increase in the wet weight of the pancreas which accompanies induction of the AHPN, and they attenuated the severity of the pancreatic pathology to such an extent that in none of the animals treated with the inhibitors was the pancreas hemorrhagic. The inhibitors attenuated also the severity of the liver and fat tissue pathology and, more significantly, pepstatin and chymostatin afforded the survival of some of the animals beyond the 5th day. Furthermore, none of the animals treated with the inhibitors, irrespective of their time of death, developed the typical shocklike state that precedes death. These effects of the inhibitors are the more remarkable and significant if one considers that they were evidenced in animals fed the experimental diet throughout, up to the time of their death or killing. Leupeptin, pepstatin, chymostatin and antipain are small peptides that have no side-effects and have been shown to also attenuate the severity of other experimental disease states. Therefore, their possible use in the therapy of hemorrhagic pancreatitis and other forms of pancreatitis appears to deserve consideration. In this regard, however, further work with other experimental models of pancreatitis may be warranted, since in the present model the induced pancreatitis is very severe and its onset often develops too rapidly to test adequately the effects of therapeutic agents.

3. Gastrointestinal Hormones: (a) Vasoactive intestinal polypeptide (VIP) is a 28 amino acid peptide that is widely distributed in mammals, in gut endocrine cells and nerves of the autonomic and central nervous systems. Histological and radioimmunological studies have established that VIP-containing neurons are present in greatest density in the submucosal and Auerbach's plexuses with some neurons penetrating the mucosa and terminating near the base of epithelial cells. The innervation by these so-called 'VIP-ergic' fibers appears to be intrinsic. This pattern of distribution suggests that VIP released from these nerves could be expected to affect gut motility and secretory function since VIP alters small bowel and colonic motility and is a potent secretagogue.

If VIP serves in some manner as a neuromodulator of gut function, it should be possible to stimulate its release by physiological stimuli. Therefore, Drs. Gaginella and Hubel (K04 AM 00471, R01 AM 28299, and R01 AM 18769) at Ohio State University and the University of Iowa studied in vitro the release of VIP in response to depolarization of the intrinsic nerves of the rabbit ileum, a tissue which secretes chloride in response to both electrical field stimulation (EFS) and exogenous VIP. EFS parallel to the muscularis propria was found to cause a significant increase in VIP concentration in the buffer bathing the serosal surface of full-thickness ileum. This effect was blocked by 10^{-7} M tetrodotoxin, a neurotoxin known to prevent neural release of transmitter from nerves innervating smooth muscle of a variety of organs from different species. When circular and longitudinal muscle was removed, the amount of measurable VIP in the tissue decreased to about one-half that of full-thickness ileum, and EFS no longer caused release of VIP into the serosal or mucosal buffers. These data indicate that EFS of rabbit ileum causes release of VIP, presumably from VIP-containing nerves present in the tissue. These results support the idea that VIP may be a physiological neuroregulator of intestinal function.

4. Salivary Glands

(a) The role of the autonomic nervous system in the regulation of salivary secretion is well established. In general, mammalian salivary glands respond to stimulation by autonomic nerves or by drugs that mimic their action. Salivary secretions can also be elicited, at least in some species, by certain peptides, particularly some that are isolated from the skin of non-mammalian species, such as physalaemin and eledoisin. Substance P is an undecapeptide which has been shown to be present in the mammalian central nervous system and in peripheral nerve endings supplying several tissues, including the intestine and salivary glands. Although it has been known for some time that substance P can induce salivation in rats, no evidence is available concerning: 1) the characteristics of the in vivo secretory response of specific salivary glands, particularly in terms of its time course and of the volume and composition of the saliva secreted, and 2) the similarities or differences that may exist between this response and that elicited by autonomic stimulation. Since substance P is present of salivary and other digestive secretions, either as a hormone or as a neurotransmitter. A better understanding of this potential role can only be ascertained, however, by a more detailed assessment of the effects of this naturally occurring peptide on secretory function. The present study by Dr. Martinez et al. (R01 AM 18150) at the University of Missouri School of Medicine, Columbia, Missouri was undertaken, therefore, to assess: 1) the direct effect of substance P on fluid and electrolyte secretion from the rat submandibular gland, and 2) its influence on the secretion elicited from this gland by autonomic agents. Intravenous infusions of substance P were found to cause a moderate but transient secretory response from the gland. The electrolyte composition of the saliva elicited by this peptide was qualitatively similar to that of the secretion induced by parasympathomimetic agents. Substance P inhibited the secretory response to acetylcholine and isoproterenol, but did not modify the binding of specific ligands to glandular autonomic receptors. It is concluded that this naturally occurring peptide plays a role in the physiologic regulation of salivary secretions, which involves both direct stimulatory effects and a modulating action on the effect of autonomic neurotransmitters on the salivary gland cells. The latter may involve similar effects of substance P and the neurotransmitters on a metabolic pathway localized beyond receptor activation.

NUTRITION PROGRAM

Gerald F. Combs, Ph.D., Program Director

The Nutrition Program, NIADDK has continued to foster and support research and training in the broad areas of fundamental and clinical nutrition in order to advance knowledge concerning 1) the function and requirements of nutrients, 2) the relationship of diet (and nutrients) to health and disease, and 3) the prevention and treatment of diseases through the application of nutrition research. The fundamental research supported by the Nutrition Program generally has been nutrient-centered rather than focused on a particular disease, organ or life cycle state, while the clinical investigations usually are related to problems affecting the nutritional status of the whole individual, such as obesity, parenteral nutrition, anorexia nervosa and combined nutrient-drug management of patients. Discoveries from basic research have advanced our knowledge of the function of essential dietary nutrients in the whole body and have found wide application in clinical medicine.

The common occurrence of nutrition-related diseases or conditions such as obesity, diabetes, osteoporosis, alcoholism, anemia and atherosclorosis in segments of the U.S. population attests to the need for a better understanding of the role of nutrients in these clinical problems. The greatest promise for solutions to such problems lies in fundamental research on mechanism of action of specific nutritional factors, and nutrient interactions, as well as the role of diet or nutrients as metabolic regulators and as physiological modifiers of behavior. Recent technological advances, including mass spectrometry (using stable isotopes), high pressure liquid chromotography, radioimmunoassay and rapid analysis of amino acids and trace metals, offer promise for rapid progress in such basic nutrient-focused studies of importance in human health.

The Nutrition Program continued to stress the following priority research areas:

1) Fundamental studies on the metabolic role of specific dietary components, including essential amino acids, protein, vitamins, minerals, essential fatty acids, fats, carbohydrates and metabolizable emergy.

2) Investigation of the underlying causes of obesity, with the aim of developing appropriate methods for prevention and control.

3) Studies on nutritional requirements in health and disease with emphasis on individual and environmental differences.

4) Studies on the nutritional support of the patient as it affects nutritional status of the whole individual with such conditions as obesity, surgical trauma, burns, or chronic renal failure.

5) Basic studies on the role of "dietary fibers" on transit time digestion, rate of absorption, intestional microflora, and interactions with nutrients, drugs, bile salts, and other substances.

6) Research on nutrition and infection and immune competence.

A significant amount of nutrition related research was supported by other NIADDK programs, especially Digestive Diseases, Diabetes, Metabolic Diseases, Hematology, Kidney Diseases, Endocrinology and Musculoskeletal Diseases Programs.

Clinical Nutrition Research Units (CNRUs)

The NIH initiated a new multi-institute program designed to foster inderdisciplinary research, training and education in human nutrition, involving core grants for Clinical Nutrition Research Units. The Nutrition Program supports five of the seven CNRUs presently active. These are located at:

1) University of Chicago (Dr. Irwin Rosenberg)
2) University of Wisconsin (Drs. Alfred Harper and Earl Shrago)
3) Vanderbilt University (Dr. Harry Greene)
4) Medical College of Georgia (Dr. Elaine Feldman)
5) Columbia University (Dr. Myron Winick)

Research in human nutrition is truly interdisciplinary and complex, being dependent on the close interactions of several basic research disciplines for breakthrough in fundamental knowledge and on the appropriate medical specialties for rapid integration into clinical studies. The CNRU awards appear to be stimulating progress in multidisciplinary research in clinical nutrition, enhancing patient care, strengthening training environments and generating nutrition information for the public.

The second annual meeting of the CNRU Directors was held on December 17, 1981, to explore potential opportunities for institutional collaboration in the areas of 1) laboratory methodology and services, 2) education and outreach activities and 3) clinical research. A meeting of the CNRU Directors, and Nutrition Center Directors from USDA, VA, FDA, DOD, and NIH has been planned in conjunction with the Joint Subcommittee on Human Nutrition Research for December 16-17, as a means of exchanging information between governmental agencies with a view of greater collaboration.

In collaboration with NCI, the site visit evaluation of all of the CNRUs after 20 months of operation will be completed in July, 1982. Representatives of USDA and VA Nutrition Laboratories were invited to participate in the evaluations conducted in FY 82. Upon completion of the site visits, an overall report will be prepared, evaluating the effectivness of the CNRU mechanism.

Program Announcement

NIADDK jointly sponsored two program announcements in nutrition during FY 82: a) Studies on Nutritional Support of the Patient, NIH Guide for Grants and Contracts, Vol 11, No 3, February 26, 1982. b) Research Grant Support in Nutrition: Environmental and Host Factors Affecting Nutritional Requirements, NIH Guide for Grants and Contracts, Vol 10, No 12, November 6, 1981.

Research Training

NIADDK continued to have a primary responsibility for research training through fellowships and training grants. During FY 82 the Nutrition Program administered 17 active fellowships and 11 training grants (20 postdoctoral and 33 predoctoral slots) in nutrition. Funds for nutrition training continued to be limiting. Four K04's and 2 K08's also were supported.

US-Japan Malnutrition Panel

The NIADDK has continued to administer the US Malnutrition Panel of the US-Japan Cooperative Medical Science Program. In order to minimize foreign travel, the annual joint meeting and workshop of the US and Japanese Malnutrition Panels was deferred until December 6-8, 1982, in Tokyo. Planning for the workshop on "Subclinical Iron Deficiency" has been completed.

The research fostered under the US and Japan Malnutrition Program is targeted primarily at those problems, and solutions, which will most benefit the undernourished of Asia. The most common states of undernutrition are protein-energy malnutrition and iron and vitamin A deficiencies. Superimposed are various diseased states, particularly diarrheal infections, which cause impaired utilization and loss of nutrients and constitute the primary cause of death of children under 5 years of age. Iron deficiency, which is widespread, is believed to interfere with immune response, cause lowered resistance to infection and reduce the functional physical capacities and possibly impair mental and learning processes. Vitamin A deficiency in turn causes many thousands of cases of damaged vision and total blindness in young children each year.

During FY 82 the number of active research grants which relate to the US-Japan Malnutrition Program has increased to 27, or 69% over the number active in FY 81. This increase is due largely to the identification of more NICHD grants that were relevant to the US-Japan goals, although the early effects of the recent workshop designed to stimulate collaborative research and the program announcement concerning environmental and host factors affecting nutritional requirements are evident as well.

Workshops

In FY 82, the Nutrition Program provided partial support and/or participated in the following workshops or conferences:

a) Conference on Vitamin E: Biochemical Hematological and Clinical Aspects
November 11-13, 1981, NYC.

b) Protocols - Mineral/Element Analysis of Human Tussies.
October 9-10, 1981, St. Louis, MO

c) Fifth Workshop on Vitamin D
February 14-19, 1982, Williamsburg, VA

d) Energy Utilization in Biological Systems
August 8-13, 1982 Food and Nutrition Workshop, Gordon Research Conference

e) Workshop on Body Weight, Health and Longevity
January 25-26, 1982, Bethesda, MD

f) Nutritional Therapy in Chronic Renal Failure Conference
April 29-30, 1982, Bethesda, MD

g) Workshop on Classification of Obesities
(to be held October 17-19, 1982 at Poughkeepsie, NY)

Nutrition Advisory Group

A Nutrition Advisory Group, chaired by Dr. Theodore Van Itallie, and composed of seven scientists well known for their nutrition research contributions, was convened by the Director to reconsider the mission of the Nutrition Program, NIADDK. The Advisory Group, in its report to the Director, stressed the major contributions of the NIADDK Nutrition Program in 1) supporting fundamental and applied primary nutrition research, 2) initiating and sustaining support of training programs in fundamental and clinical nutrition and 3) the creation and support of Clinical Nutrition Research Units.

The Advisory Group also recommended that a Center for Nutrition be created within the NIADDK with the charge of:

1) identifying research and training needs in primary nutrition,

2) developing initiatives relating to training and research in nutrition science, both intramurally and extramurally and

3) establishing a broad Nutrition Advisory Committee at NIH.

It was recommended also that an established clinical nutritionist be recruited to head this Center for Nutrition.

Research By Contract

The third year of the five year contract with the Food and Nutrition Board, National Academy of Sciences (NO1 AM 0-2204) was renewed at a cost of $79,000. This contract initiated, December 15, 1979, is entitled "Preparation of the Tenth Edition of Recommended Dietary Allowances and Related Studies". The revised RDAs are to be completed in 1985. Guidelines for the use of the RDA is to be prepared for publication in 1983. These activities are proceeding on schedule. The Committee on Dietary Allowances and its subcommittee on uses of the RDAs have held several working meetings using the committee study approach. A draft report has been prepared on guidelines for uses of the RDAs.

Trends in Extramural Research

OBESITY

Despite the recent emphasis on obesity research, the prevalence of obesity and associated mortality appears to be increasing in the US. A recent survey revealed that 4.9% of the men and 7.2% of the women aged 20-74 years were severely obese, representing 37 and 57 % above ideal body weight in all age groups, respectively. Obesity seems to have clinical significance at about 30% over ideal weight for sex and height in the adult even in the absence of hypertension, hyperlipidemia or diabetes. The increase in prevalence of obesity is attributed in part to decreased physical activity and energy expenditures, and increased ability of highly palatable foods.

Recent changes in life styles and a marked increase in the availability of highly processed food items, including more convenience foods, soft drinks and snacks, has led to corresponding changes in food patterns of per capita consumption. Per capita consumption of cereal products has halved since 1910. Widespread use of refined flour has considerably reduced the bulk and dietary fiber content. Refined sugar consumption per capita increased steadily up to 1950 but has changed little since that time. Nevertheless, about 16% of our food energy today comes from refined sucrose.

Consumption of red meats, especially beef, has essentially doubled in the past fifty years. Meat products are generally high fat, high energy, low residue foods. Intake of vegetable oils also has increased. Consumption of fruits, excluding juices, has decreased by one-forth since 1952. The long term trend shows that fat intake per person has increased 24%, carbohydrate intake decreased 25%, while protein and energy intake have remained about the same since 1909-1913.

Highly processed foods generally have a reduced content of vitamins and minerals, especially trace minerals. Usual milling of wheat flour results in a loss of approximately 70-80% of most mineral elements originally found in wheat. Similiarly, losses for most of the vitamins amount to approximately 50-80% in milling wheat. These losses of dietary essential trace elements or vitamins in diets composed of highly milled, refined foods may have major health significance, esentially when it is realized that dietary requirements are not established for a number of these nutrients. The possible effect of *suboptimal* intakes of specific minerals (such as zinc, iron, copper, selenium, chromium, magnesium) on physiological appetite and the tendency of man to over eat and become obese appears to deserve priority research attention.

A recent report by Grinker (1981) reveals that the belief that obesity is primarily the result of a drive to consume highly palatable foods (a psychological aberration which overrides any biological regulation) is a misconception. On the contrary, lean and obese humans and rats showed evidence of physiological control of food intake. This control is evident on the basis of daily caloric intake and meal size, and is responsive to changes in palatability, caloric concentration and other factors (R01 AM 27980). Grinker and Bellisle (1981) also did not observed reduced voluntary food intake in human subjects when plasma glycerol levels were elevated by glycerol preloads prior to eating, suggesting the it is highly unlikely that glycerol, or glycerol in relation to FFA levels, is a specific satiety signal.

Faust (R01 AM 20508) in repletion studies following starvation in rats, using a radioactive labeled DNA precursor (^{3}H-thymidine), found no evidence of adipocyte turnover, confirming the previous observation that extreme food deprivation does not alter fat cell number in the rat. Faust found that castration of 4-month-old rats greatly accelerated adipose tissue regeneration following lipeatomy at 10 months of age. Faust also has identified two different fibroblast-like cells from cultured adipose tissue stromal-vascular cells. One of these is an adipocyte progenitor which differentiates to become an adipocyte. Cultures from young rats show many lipid-filling cells, while similiarly produced cultures from older rats do not. Moreover, parametrical deposits of female rats fed a high fat diet failed to show increases in fat cell number *in vivo* for many months.

Cultures of stromal-vascular cells from deposits of such rats (taken after several weeks of high-fat feeding) also show many fewer lipid-filling cells than similiar cultured cells from controlled rats. This technique may prove to be useful in assessins the proliferative potential in adipose tissue samples taken from people.

Keesey (R01 AM 19944) in collaboration with Stern, found that lateral hypothalmic lesions in genetically obese (fa fa) Zucker rats caused both the adipose cell number and size to be reduced to levels closer to those of lean litter mates than those of obese controls. Neither the lean or obese LH-lesioned rats displayed an increse in cell number when fed a palatable diet which markedly increased carsass lipids. These and the observations 1) that lean control rats maintained a higher body weight than LH-lesioned rats to which they were pair-fed and 2) that in obese rats, food restriction further reduced protein deposition and elevated plasma insulin relative to comparably fed LH-lesioned obese rats, suggest that the LH-syndrome is not mimicked by simple food restriction. Thus it appears that lateral hypothalamic lesions produce their effects by affecting a mechanism which sets the regulated level of adiposity and possibly adipocyte number. In another study the resting metabolic rate of ventromedial hypothalmic (VMH) lesioned rats, were from 10-18% less that the non-lesioned controls, when maintained at similar body weights by limiting their access to food. This pattern in VMH lesioned rats of reduced energy expenditure at comparable body weights suggests that altered energy utilization and expenditure play an important role in obesity produced by this lesion.

McHugh (R01 AM 19302) in studies with rhesus monkeys, found that refilling the stomach with the same volume of non-nutrient saline in place of the gastric contents following a 15 minute drinking bout exerted a marked reduction on subsequent glucose intake as compared to that observed when the stomach contents were removed and the stomach was allowed to remain empty. These results suggest that gastric distension per se is a potent satiety signal.

Ritter (R01 AM 20035) obtained results in rats with microinfusions of 5-thioglucose into the lateral or forth ventricle of the brain that suggest that the glucoreceptors that mediate feeding behavior and hyperglycemia in response to glucoprivation are located in the caudal hindbrain and not in the hypothalamus where they have been sought previously.

Studies in rats by Gibbs (AM17240) showed that the satiety effect of intraperitoneal bombesin (BBS) is unaffected by abdominal vagotomy even though the satiety effect of the octapeptide of cholecystokinin (CCK) was abolished. Thus, BBS and CCK produce satiety through different mechanisms, and BBS does not produce satiety by releasing endogenous CCK. In another study, Gibbs found that intravenous infusions of CCK-8 significantly decreased food intake in both obese and lean men (12-13% less as compared to saline controls), indicating that obese men are as sensitive to the satiety effect of CCK-8 as non-obese subjects.

Woods (R01 AM 17844) injected baboons with bombesin which reduced voluntary food consumption at low doses (1 ug/kg), but basal immunoreactive insulin (IRI) levels were increased slightly while post-parandial increases of IRI were partially supressed. Gastrin releasing hormone (GRH), a mammalian BBS, however caused a dose-related suppression of meal size in rats and elicited the complete behavioral sequence of satiety.

In studies on brown adipose tissue (BAT), Glick (R01 AM 27019) has obtained data that strongly suggest that BAT plays an important role in the overall expression of the thermic effects of single meals in the rat. Respiration rate of BAT following a high fat meal was reduced in comparison with a high carbohydrate meal, while protein level had no significant effect. Brown adipose tissue was found to have a short latency response to nutritional state of rats, as single meals resulted in large changes in size, composition and metabolic activity of BAT. Glick has consistently associated satiety with decreased heat production of brown adipose tissue. This finding may suggest the existance of thermoreceptors in BAT linked with the control mechanism of food intake.

Danforth (R01 AM 18536) is studying the nutritional-induced changes in the metabolism of thyroid hormones and catecholamines in relation to changes in thermogenesis.

Romsos (R01 AM 15847) used norepinephrine turnover rates in various tissues as an indicator of the sympathetic nervous system activity and its relationship to obesity in rats. Weanling VMH-lesioned rats were used; such rats do not over eat, but become obese due to lowered energy expenditure. In such VMH-lesioned rats, norepinephrine turnover rates were lowered 38% in brown adipose tissue. This change in autonomic tone is very likely to be a major factor in the development of obesity in the VMH-lesioned rat.

Kanarek (R01 AM 19821) found that rats provided a choice of sucrose, glucose or fructose solutions and a standard diet had significantly more brown adipose tissue that rats fed only the standard diet. This effect was greatest with surcose.

Oscai R01 AM 17357) observed that exercise in rats increased endogenous lipoprotein lipase and decreased intracellular triacylglycerols in heart and skeletal muscle. These results suggest that lipoprotein lipase may be the enzyme responsible for the intracellular hydrolysis of triacylglycerols in muscle during exercise.

In studies by Allred (R01 AM 19555) on the mechanism by which diet controls fatty acid biosynthesis, hormonal regulation of acetyl CoA carboylase was found to involve phosphorylation-dephosphorylation of this rate-limiting enzyme of fatty acid synthesis. The results suggest that more than one enzyme can catalyze the phosphorylation of acetyl CoA carboxylase and thereby decrease its activity.

In addition, the results indicate that acetyl CoA carboxylase itself may be altered by nutritional status.

Schoeller (R01 AM 30031) has initiated studies to validate the doubly labeled water method for measuring energy expenditure. In this non-invasive method, water made from the stable isotopes, ^{2}H and ^{18}O, is administered and the excretion of ^{2}H lost in $^{2}H_2O$ and ^{18}O lost as $C^{18}O_2$ is measured. Preliminary results agreed well with the measured intake and had a coefficient of variation of 6%. Comparisions will be made using indirect calorimetry as a reference method.

Minerals

It is likely that trace mineral inbalances or inadequacies may be responsible for certain metabolic adaptations, some of which may lead to chronic metabolic disease. Iron and zinc status of certain segments of the population reflect inadequate dietary intakes. Some concerns have been expressed recently about the adequacy of diets with regard to copper, chromium, selenium, and perhaps other mineral elements. Trace mineral nutrition research offers great promise, since so little is really known about the practical importance of these elements in relation to human health. The Nutrition Program supports research on iron, zinc, selenium, copper, silicon, chrominum, tin, aluminium, molybdenum, magnesium, calcium and phosphorus.

Iron deficiency remains on the the most common forms of malnutrition and is particularly prevalent in rapidly growing infants and children. Dallman (R01 AM 13897) observed that skeletal muscle cytochrome C and myoglbin were depressed especially in young animals fed iron deficient diets. Humans with iron deficiency anemia have diminished exercise capacity and show evidence of decreased productivity. Using exchange transfusion to control hemogolbin concentration in iron-deficient and control rats, both brief hard exercise and prolonged submaximal exercise were found to be impaired by muscle iron deficiencies. Transfusion with packed red cells largely corrected the VO_2 max and aerobic work capacity but did not significantly impove the endurance capacity of iron deficient animals. These results suggest that defects in VO_2 max and aerobic work capacity in iron-deficient rats result primarily form impaired oxygen delivery, whereas the loss of enduramce capacity is a direct function of the diminished capacity of muscle mitochondria to utilize ozygen and produce ATP. Hence, treatment of iron deficiency will reverse impaired endurance in prolonged exercise and anemia at different rates.

In other studies, using the laser diffraction viscosimeter to study the deformability properties of iron deficient red blood cells and RBC membrane ghosts, Dallman found that the decreased red cell deformability was due primarily to decreased membrane viscoelasticity. Also biochemical studies showed that iron deficient red blood cells had increased susceptibility to peroxidation and an alteration in cation content. In addition, iron deficient rat RBC's showed evidence for spontaneous membrane lipid and protein cross linking. It is suggested that increased RBC membrane stiffness in iron deficiency may be related to peroxide damage and is responsible for the decrease in red cell survival observed.

A "small dose iron tolerence test" has been developed by Crosby (R01 AM 27642) involving oral administration of 5 to 30 mg of iron as sulfate or fumarate and measuring plasma iron concentrations at intervals for eight hours. Iron replete men show no change in plasma iron, while mildly iron deficient subjects exhibit significant increases in plasma iron concentrations during the first several hours. The shape of the iron tolerence curves appear to vary with differences in total body iron status. This new method promises to be useful in studying, without radioactivity, both nutritional aspects of iron absorption and the movement of iron through the plasma.

Metabolism of iron in normal and iron-depleted tissue in rats was studied by Hegenauer (R01 AM 12386) in order to link work performance and insufficient iron at the cellular level. By using tracer amounts of ^{59}Fe given orally, it was found that the major organ accumulation was the spleen, but significent amounts were also localized in heart and brain. In muscle tissue the ^{59}Fe entered the mitrochondria. Enhanced mitochondrial uptake of iron prior to any detectable change in hemoglobin level may be indicative of biochemical changes and decrements in work capacity. In other studies mitochondria from heart and striated muscle retained their iron more tenaciously under stress of iron deficiency than mitochomdria from liver and spleen.

Since the report of Prasad in 1958, suggesting that dwarfism and hypogonadism observed in Iranian boys might be due to a nutritional deficiency of zinc, investigators have observed zinc deficiency in groups of people in widely different countries. Hambidge (R01 AM 12432) conducted a long-term double-blind study in Denver in matched children (ages 3-6 years) below the 10th percentile for height and showing borderline zinc nutrition. Linear growth of the 13 pairs of boys who have completed the study show that zinc supplementation (4.3 mg/day) significantly increased the growth velocity in males. No significant differences were observed in levels of hair zinc, plasma zinc, serum alkaline phosphate activity or serum vitamin A between test and control groups. Appetite, as measured by dietary intake, also was decreased in the children not receiving supplemental zinc. The results indicate that low zinc intakes can limit skeletal growth rates of rapidly growing young children. In other related studies, the bioavailability of zinc added to human milk was found to be better that that of zinc added to bovine milk. In addition, two 9-week-old breast fed premature infants, were observed who developed acrodermatitis and hypozincemia because of low zinc content in the breast milk. All symptoms of zinc deficiency disappeared within seven days after the infants were treated orally with zinc. Zinc secretion into breast milk appears to be a controlled process independent of zinc intake and serum level.

Work by Freeland-Graves (R01 AM 26875) has revealed that the activity of a zinc dependent enzyme, RNAase, was significantly increased in the plasma and parotid saliva in young adult men fed a zinc deficient diet for 8 weeks, and returned to normal with zinc repletion. The zinc depleted men also exhibited leukopenia with relative lymphocytosis, which may relate to the role of zinc in immune function. The sensitivity of the RNAase enzyme in two biological fluids suggest that it may be an appropriate enzyme for monitoring zinc status in man.

In more basic studies by Cousins (R01 AM 31651), primary cultures of adult rat liver parenchymal cells were maintained as a monolayer to investigate the characteristics of zinc accumulation and metabolism. Accumulation was found to be a temperature dependent, saturable process inhibited by cadmium and inhibitors of energy production (cyanide, azide). Glucocorticoids specifically stimulated accumulation as a function of potency. The additional complement of zinc was accumulated as newly synthesized metallothionein. Zinc accumulation as metallothionein also can be induced by increasing the extracellular zinc concentration above physiological levels.

Tappel (AM 06424) has purified glutathione peroxidase containing ^{75}Se and determined the amino acid sequence. This work is of significance as it provides positive proof the selenocysteine is in the backbone of the protein and provides the first definitive data on the amino acid sequence of the "active site" portion of the enzyme. Other selenoproteins also have been found to contain selenocysteine and an active protein synthesis system for incorporation of selenium into selenoproteins has been postulated. A selenocysteyl tRNA was formed via an aminoacyl linkage, which has the qualities required to be an intermediate in such a pathway. Tappel (R01 AM 09933) has used pentane and ethane production as an index of in vivo lipid peroxidation. Vitamin E and Se were found to protect against lipid peroxidation.

McCay (R01 AM 08397) has characterized a heat-labile glutathione-dependent factor which inhibits lipid peroxidation in biological membrane. The cytosolic factor, termed peroxistatin, inhibits lipid peroxidation in membranous organelles initiated by either enzymic or non-enzymic means. It is not glutathione peroxidase nor one of the glutathione S-transferrses, although it contains sulfhydryl groups necessary for the inhibitory activity. Purification studies indicate that peroxistatin is a rather small protein with a molecular weight of approximately 15,000 daltons.

Protein and Amino Acids

Metabolic experiments conducted recently at University of California, Berkeley (King P01 AM 10202) have revealed several interesting findings: 1) Pregnant women retained about 1 gram more of protein nitrogen daily than non-pregnant women fed the same diet; 2) Zinc absorption from diets composed of usual foods averaged about 15%, determined by stable isotopes; 3) Selenium depletion in human subjects induced a negative nitrogen balance and loss of lean body mass; 4) Self selected diets adequate in energy and protein provided less zinc and copper than recommended; 5) When young, healthy, active men on "prudent" diets decreased their energy flux from 10 miles to 5 miles running per day, their mean levels of low density lipoproteins increased by 17% and high density lipoproteins decreased 13%, changes associated with increased risk of atherosclerosis, and 6) A diet change resulting from limiting the protein-rich animal foods and prohibiting refined carbohydrates and fats in an otherwise usual calorically dense diet, let to a 500 kcal decrease in energy intake per day and a 9% reduction in "on-the-feet" activities.

Using non-invasive, stable isotope probes, Young (R01 15856) has studied dynamic aspects of whole body amino acid metabolism brought about by changes in intake of dietary protein and energy. The results reveal that the synthesis of alanine is extensive and increases under conditions of low protein intake and high energy intake, suggesting that alanine plays an important role in the redistribution of body nitrogen and the recycling and retention of amino nitrogen, when there is need to conserve it. In contrast, changes in glycine metabolism do not suggest a major role for this amino acid in maintaining whole body nitrogen economy. In addition, results from this laboratory with leucine have opened the way to new approaches for estimating the amino acid requirements of healthy adults. Prelinimary data with leucine suggest that the requirement for this amino acid has been underestimated. Recently Matthews (R01 AM 25994) and Young have reported that the leucine requirement is at least 20 mg/kg/day, based on the use of stable isotopes in man.

In studies designed to determine the mechanism responsible for amino acid induced orotic aciduria, the addition of lysine to isolated hepatocytes has been shown to reduce the arginase activity as much as 90%, accompanied by a progressive

6) Relatively small variations in diet composition, given for short periods (2-3 days) were found to cause significant alterations in "normal" spontaneous behavior, i.e., motor activity in rats. As the carbohydrate-protein ratio in the diet increased, the rats were more continuously active. This effect was independent of dietary fat content, or of the total number of calories consumed. This affirms the premise that relatively small dietary changes can significantly affect behavior.

Dr. Adibi (R01 AM 15855) has studies the effect of carbohydrate intake on the modulation of leucine oxidation and turnover in obese subjects in which ketosis was induced by staravtion. The results suggest that a) ketosis directly or indirectly enhances oxidation of leucine, b) starvation-induced ketosis, proteolysis and glucoenogenesis can be prevented sequentially by carbohydrate diets providing 300 to 800 kcals per day, c) the reduced leucine turnover in starvation is most likely the result of reduced protein synthesis, and d) dimunition of branched-chain amino acid catabolism is a component of the nitrogen-sparing effect of carbohydrate.

Mitch (R01 AM 26903), using the isolated perfused rat hindquarter perparation, has shown that muscle protein synthesis is depressed about 30%, and that there is an increased rate of muscle protein degradation in rats with acute uremia. In rats with chronic renal failure, protein synthesis appears to be depressed about 10% and degradation is increased about 25% over sham-operated, pair-fed controls. These results provide insight into the abnormalities in protein nutrition associated with uremia which may help explain the apparent clinical value of leucine and its keto analogue in renal disease.

Mitch and Walser also have found the infusion of the keto analogue of leucine (alphaketoisocoproate) decrease the nitrogen wasting of starvation in obese patients, whereas leucine does not when administered under identical conditions. Other work indicates that alpha ketoisocaproate tends to prevent protein degradation while leucine promotes protein synthesis.

Walser (P01 AM 18020) also has completed a study of the short-term effects of branched-chain ketoacids administered as ornithine salts, in Duchenne's muscular distrophy. A significant reduction in the rate of muscle protein breakdown was demonstrated, although nitrogen balance did not improve.

Vitamins

Henderson (R01 AM 19012) has investigated the digestive process by which nicotinic acid or nicotinamide are released for absorption since niacin is present in foods chiefly as pyridine nucleotides. Nicotinamide is absorbed very rapidly by passive diffusion. Nicotinic acid is transported more slowly by facilitated diffusion and a greater proportion is retained in the intestine and converted to nicotinamide adenine dinucleotide (NAD). Prior to absorption NAD is largely converted to niacinamide, after the sequented action of pyrophosphatase, phosphatase, and a glycohydrolase.

Thanassi (R01 AM 25490) has observed that the metabolism of vitamin B_6 is characteristically different in Morris hepatomas from that in rapidly proliferating hepatic tissue, as the hepatomas have little or no PLP oxidase activity.

Hall (R01 AM 17767) has shown that cobalamin (vitamin B_{12}) has a role in immunoglobin production and release through Ig G and in protein synthesis in normal and dificient humans lymphocytes. The effect is not explained on the basis of cell number since protein and Ig G synthesis was enhanced by cobalamin per unit cell.

Stockstad (R01 AM 08171) has obtained results which indicate that the megaloblastic anemia, which results from exposure to nitrous oxide, is due to the fact that N_2O exposure of human tissue markedly decreases methionine synthetase, a B_{12} dependent enzyme. Studies with mice showed that recovery is rapid after N_2O is terminated.

Other studies by Stockstad include the isolation and identification of a potent unidentified folic acid antagonist. This antagonist, termed "x-methyl folic acid" contains 4 more carbons that folic acid. It possesses potent anti-folic acid activity for animals and bacteria, which might be considered for use as an anti-tumor agent in methotrexate-resistant patients.

Wagner (R01 AM 15289) has discovered that folate binding proteins of mitochondria are the enxymes, dimethylglycine dehydrogenase and sarcosine dehydrogenase. For both of these enzymes, small peptides have been isolated which contain covalently bound flavin. The flavin appears to be flavin adenine diucleotide bound to a histidine residue. In addition, a high molecular weight folate binding protein has been purified from rat liver cytosol. The native protein contains bound tetrahydropterol pentaglutamate, and consist of two idential subunits.

Halsted (R01 AM 18330) has shown that dietary folates which exist primarily in food as pteroylpolyglutamates, are abordbed in the jejunum by, a process involving hydrolysis and subsequent intestional transport of pteroylmonoglytamyl folate. At least one (or more) intestinal mucosal enzyme(s), termed folate conjugase is required for this transformation. This investigator also has determined a clear relationship between sulfasalazine and folate deficiency in patients with ulcerative colitis.

Waxman (R01 AM 16690) has reported that pteroyl polyglutamates bind to hemoglobin with an affinity that leaves only a minor fraction of red cell folate in the free state. This could serve as a regulating mechnaism for protein synthesis in the differentiating erythroblast.

Tappel (R01 AM 09933) has shown that vitamin E in selenium deficient rats protects against lipid peroxidation in rats treated with 50 mg of methylethyl ketone peroxide kg body eat as measured by pentane prodution. Chronic ethanol consumption (9 weeks) did not influence lipid peroxidation in vitamin E and Se deficient rats, even though the rats fed vitamin E had a level of pentane production one-half of that of the vitamin E deficient rats.

Rosenberg (R01 AM 26561) found that in rats, the majority of vitamin D_3 and 25(OH) vitamin D_3 was transported from the intestine (jejunal sacs) in portal blood rather than lymph, and the absorption of 25(OH) vitamin D_3 was greater than vitamin D throughout a range of luminal fat levels. These differences may explain the superior absorption of the 25(OH) vitamin D in patients with malabsorption. Using isolated jejunal segments in rats with thoracic and common bile duct cannulase, it was found that 1,25 $(HO)_2$ vitamin D_3 also is transported mainly in portal blood rather than lymph and is not dependent on chylomicron production. Bile salts increase the amount of 1,25 $(OH)_2$ vitamin D_3 in lymph, but only slightly accelerates total absorption.

Glucuronides of vitamin D_3 or 25-OH vitamin D_3 per se represent only a fraction of total biliary glucuronides; the major portion is comprised of more polar yet unidentified metabolic forms of the vitamins. Cause of osteopenia in chronic cholestosis is being studied. Serum 25-(OH) vitamin D_3 levels correlated with intestinal Ca absorption in most patients but these parameters were not predictive of the histology of metabolic bone disease in these patients.

Curtis (R01 AM 21354) has observed that in children with moderate renal insufficiency, restriction of dietary phosphorus increases the plasma concentrations of 1,25-$(OH)_2$ vitamin D, reflecting a reduction of and the suppression of activity of 25-hydroxyvitamin D-1 hydroxylase. Additional paired studies in children with moderate renal insufficiency also show that oral phosphorus loading suppresses plasma levels of 1,25-$(OH)_2$ vitamin D, despite an increase in serum levels of parathyroid hormone. These findings, together with animal data, provide strong evidence that phosphorus intake plays an important role in regulation of 1 hydrolase in the remaining functioning nephrons.

Gray (R01 AM 22014) has shown also that the pituitary is necessary for maintenance of elevated 1,25-$(OH)_2$ vitamin D plasma levels during phosphate deprivation in rats, suggesting that growth hormone is involved.

Deluca (R01 AM 14881) has shown that there are two distinct mechanisms whereby intestinal calcium transport responds to the active form of vitamin D. The first is an initial response by existent villus cells; the second is a response of crypt cells that are programmed to begin calcium transport as they move into position on the villus membrane. This laboratory also has succeed in isolating in pure form the 1,25-$(OH)_2$ D_3 receptor from intestinal mucosa of normal chicks. Four more vitamin D metabolites have been identified and extensive studies on the metabolism and function of vitamin D have been continued.

Deluca and Riggs have found not only that plasma 1,25-$(OH)_2$ D_3 is low in postmenopausal women, but their intestinal absorption of calcium also is diminished. Treatment of postmenopausal osteoporotic females with 0.5 mcg of 1,25-$(OH)_2$ D_3 per day markedly improved calcium balance, but of great importance, it increased trabecular bone volume and decreased bone fracture rate. This work illustrates the potential use of vitamin D hormone in the treatment and prevention of osteopenia of postmenopausal state. Vitamin D metabolites also are used currently in the treatment of a variety of metabolic bone diseases, including renal osteodystrophy, hypoparathyroidism, pseudohypoparathyroidism, vitamin D resistant rickets, and now osteoporosis. Hoekstra (R01 AM 14881) has shown that adriamycin, and anticancer agent, clearly stimulates lipid peroxidation, particularly in selenium and vitamin E deficient rats. It is possible that the degeneration of heart muscle, a side effect of adriamycin, might be caused by peroxidative damage preventable by higher levels of vitamin E and selenium.

Russell (R01 AM 31081) has modified a rapid dark adaptation test (earlier described by Thornton) by matching the intensities of the colored discs to cone function, instead of rod function, so that under dim lighting conditions the ability to separate the different colored discs is dependent on rod function alone. The mean time to complete this rapid test was 4.4 ± 0.8 minutes in normal adults and 7.7 ± 1.7 minutes for vitamin A deficient individuals. This rapid functional test for vitamin A deficiency, can be used under field conditions as the equipment is easily transportable and is non-invasive. The sensitivity of the rapid test (± 1.8 standard deviation) was 95% for vitamin A deficiency. The method has been tested with 4 year old children in Baltimore and Guatemala and found to be very satisfactory. This rapid dark adaptation test is expected to be widely used in population surveys to identify subclinical vitamin A deficiencies.

Dietary Fiber

Schneeman (R01 AM 20446) has shown that fiber sources, such a cellulose or wheat bran, can cause lower levels of digestive enzyme activity in the small intestine by dilution of the contents or by interfering with enzymatic activity as seen in vitro. Dietary wheat bran resulted in an elevation of digestive enzyme activity in the pancreas. Dietary fibers such as pectin and cellulose led to accumulation of material in the small intestine. Human pancreatic enzyme activity was altered in vitro by incubation with dietary fiber sources. These changes in the gastrointestinal tract can be expected to result in differences in the rates of digestion and absorption.

Oace (R01 AM 19970) has found that the depressed bioavailability of vitamin B_{12} resulting from pectin in the diet, cannot be explained simply by interference with absorption due to calcium binding. This laboratory has also shown that a bacterially digestible dietary fiber, (xylan) increased fecal and liver folate levels beyond that provided by the diet, indicating enhanced bacterial synthesis of folic acid in the gut.

In view of the interest in dietary fiber as a dietary component and as a therapeutic agent, and in view of the health problems which may be associated with consuming a fiber-depleted diet, basic research is necessary to determine the effect of fiber on digestion and absorption of components of the diet. To make knowledgable recommendations for the consumption of fiber we should have a better understanding of the basic mechanisms by which it affects metabolism. The effects of fiber on metabolism which have been reported could be related, in part, to alterations in the rate of digestion and absorption of nutrients from the small intestine.

National Digestive Diseases Education and Information Clearinghouse

Billie B. Mackey, Director

The National Digestive Diseases Education and Information Clearinghouse is an information service of the NIADDK. The Clearinghouse, in response to the National Digestive Diseases Commission recommendations, acts as the coordinator of a national effort to educate the patients, their families, physicians and other health care providers as well as the public about the prevention and management of digestive diseases.

Program objectives for the Clearinghouse are: to provide a central point for the exchange of information among professional organizations, foundations, and voluntary health organizations involved with digestive health and diseases, to aid the distribution of available education materials, to identify needed informational and educational materials, and to encourage production of these needed materials.

In January, 1979, the Digestive Diseases Commission made its report to Congress. The first recommendation made by the Commission Report was to establish a National Digestive Diseases Education and Information Clearinghouse under the NIADDK. The Commission Report also outlined specific functions for the Clearinghouse.

In response to the Digestive Diseases Commission recommendations, the Institute began the development of the Clearinghouse in early 1980. In consultation with the digestive diseases professional and lay organizations who named advisors to help the Clearinghouse, plans were made for the focus and activities of the Clearinghouse.

In December 1980, the Health Programs Extention Act of 1980 (S988, Section 440) mandated the Clearinghouse as follows.

> "The Secretary, acting through the Director of the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, shall establish an information and education center for digestive diseases (1) to identify, collect, analyze, and disseminate information respecting the diseases, and (2) to serve as a national educational resource for patients with such diseases and their families, physicians, and other health professionals".

Two meetings have been held this year with the advisors to the Clearinghouse in which plans for the Clearinghouse have been developed, and attainable goals for the second year of operation have been identified.

Four fact sheets on the following topics were produced, "Gallstone Disease" written by Malcolm P. Tyor, M.D. of Duke University Medical School, "Bleeding in the Digestive Tract" written by Paul Sherlock, M.D. of Sloan Kettering Cancer Research Institute, "Milk Intolerance" written by Gary Gray, M.D. of Stanford University Medical School, and "Constipation", written by Marvin Shuster, M.D. of Baltimore City Hospital.

Development of future fact sheets has been ongoing with manuscripts in progress on the following topics: "Stomach Ulcers", "IBD", "Common Digestive Diseases", and "General Digestive System". Other topics have been recommended for future fact sheets on "Irritable Bowel Syndrome", "Indigestion", "Dietary Fiber", and "Yellow Baby".

Notes on topics such as "Sucralfate Approved for Duodenal Ulcer", "Study Results Outline Treatment for Crohn's Disease", "New Vaccine to Combat Hepatitis B", and "The Hydrogen Breath Test: What It Is and How It Works" have been produced. A new flyer describing the Clearinghouse was designed, the "Directory of Organizations" was updated, and a "Glossary of DD Terms" was developed.

Short, five minute interviews with three of the fact sheet authors were filmed on videocassette for showing at the NIADDK Exhibit booth during Digestive Diseases Week in Chicago, May 16-19, 1982. This film will be available on loan for physicians who are interested in showing it to their patients.

Distribution of materials is primarily through professional and lay organizations. The Clearinghouse collaborated with the lay digestive diseases organizations for distribution of material to their chapters and members. These Clearinghouse education materials included the fact sheets "Cirrhosis of the Liver", "Diarrhea: Infection and Other Causes", and "Heartburn" as well as the flyer, the "Directory of Organizations", and the "Letter" from the Clearinghouse. A mass mailing was made to the membership of professional organizations through the Federation of Digestive Diseases Societies, to the American Medical Writers Association, and to the NIH mailing list of science editors of newspapers.

A plan for evaluating patient education materials currently available was designed and (with a few exceptions) the materials within the scope of the Clearinghouse have been evaluated.

ANNUAL REPORT FOR THE

DIVISION OF KIDNEY, UROLOGIC AND HEMATOLOGIC DISEASES

OFFICE OF THE ASSOCIATE DIRECTOR - Dr. Nancy Boucot Cummings

The Division of Kidney, Urologic and Hematologic Diseases (KUHD) of the National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases (NIADDK) places primary emphasis upon research into basic mechanisms of organ and tissue function and into the diseases of the disciplines of nephrology, urology, and hematology for which KUHD bears responsibility. The goal of the KUHD research support is development of an understanding of the physiology and pathophysiology of the kidneys, urinary tract and blood forming systems so that rational treatments and means of prevention and/or arrest of these diseases may be devised. Over ninety per cent of the funds within the KUHD Division are awarded for investigator-initiated research projects while the remaining funds fall within research career development and training, and a very modest and diminishing amount in the contract area.

With increasing Congressional, and Departmental, as well as public, concern about the increasing costs of the End Stage Renal Disease (ESRD) Program, increasing efforts have been undertaken to study, delineate, and recommend possible relevant avenues for future research. A major contribution of time and effort has been made to the Intradepartmental ESRD Strategic Work Group Report. This report recommended, in descending order of priority, that the HCFA Medical Information System (MIS) be improved in order to facilitate epidemiological and cost analyses of ESRD; that prevention be emphasized; and that research related to causes of ESRD be emphasized in order to improve knowledge to prevent, since few of the casual diseases have preventive measures possible; increased biomedical research; and measures to facilitate transplantation and dialyzer reuse. A conference on Nutritional Therapy in Chronic Renal Failure was held which provided a summation of the state of the science and therapy in this area. As an outgrowth of this conference, and utilizing recommendations of the study, Research Needs in Nephrology and Urology, and of the Ad Hoc Advisory Committees which have met over the past three years, a plan for metabolic/nutritional research in chronic renal disease was drawn up, part of which was included in recommendations to the Office of the Assistant Secretary for Health.

The Workshop on Reuse of Consumables in Hemodialysis, which focussed especially on dialyzer reuse was held March 1-2, 1982. Originally coordinated by the National Center for Health Care Technology, and held under sponsorship of a consortium, this meeting addressed a multiplicity of issues on this topic, including results of the NIADDK supported contract on Multiple Reuse of Hemodialyzers. The Continuous Ambulatory Peritoneal Dialysis (CAPD) Registry which was supported under contract to the University of Missouri had its pilot registry phase completed April 15, 1982, and has entered the phase of the National Registry. The Advisory Committee to this contract is monitoring its progress closely and continues to provide the NIADDK advice about directions, including potential utilization of the registry data and possible additional studies to be undertaken, as well as quality assurance, by site visits.

The Subcommittee on Intergovernmental Relations and Human Resources of the Committee on Government Operations held hearings about the ESRD Program, Part 2, which focussed on NIH/NIADDK/KUHD/Chronic Renal Disease Program and its research relevant to ESRD, especially in the areas of CAPD and of nutritional therapy, and the NIH interface with the Health Care Financing Administration (HFCA). The strong committment of the KUH program to conduct research into the causes and complications of ESRD in order to treat, prevent, or arrest the responsible diseases was emphasized.

Efforts to bring new scientific disciplines and technical advances into kidney and urologic research are being made. Presentations relevant to this have been made by talks, workshops, and panels presented to professional groups including the Society of University Urologists, the American Society of Pediatric Nephrology, the American Urologic Association, several university nephrology departments, and the National Kidney Foundation. In order to encourage cross disciplinary research and to introduce disciplines such as genetics, cell biology, metabolism, biochemistry, immunology, etc., into nephrology and urology, it has been suggested to training program directors that efforts be made to include such subjects in their programs. KUH program announcements will include similar recommendations. Because of the paucity of investigators in urology, innovative ways to encourage research training are being sought in this field.

Research Needs in Hematology is in the stage of final editing and will be printed early in FY 83. With continuing KUH research interest in Cooley's anemia, toxicologic testing of appropriate iron chelators in animals has been added to the contract effort for development of iron chelating agents. Major advances in the field of hemoglobin genetics, gene sequencing, and prenatal testing contribute not only to fundamental knowledge in hematology but also to the potential for treatment of inherited hematologic disorders.

Major efforts continue to coordinate and to collaborate with other institutes of NIH with whom the KUH Program has complementary and/or overlapping interests; with other governmental agencies; with outside lay and professional groups; and with scientists in the respective fields. In consultation with scientists in the field, continual evaluation and assessment of the research supported and of other aspects of program activities are carried on so that areas requiring new emphases may be approached, within the limits placed by available funding.

Through interagency committees, coordination with other groups holding related interests continues. An informal NIH Urology Coordinating Committee has been formed which includes six institutes with complementary interests in various aspects of the discipline. A similar coordinating committee for chronic renal disease is being planned. A presentation about artificial organs provided an overview of all NIH research in this areas, another area of complementary interest and informal coordination.

The funds administered by the Kidney Disease and Urology Program during FY 1982, including the support for investigator-initiated research grants, R01s, R13s & R23s (289), program projects (10), contracts (6), Clinical Investigator Awards (12), and Research Career Development Awards (19) exceeded $ 38.0 million, while the support for National Research Service Awards, both individual (20) and institutional (30) approached $2.0 million.

Manpower Development

Five competitive renewal, and one new supplemental institutional National Research Service Awards (T32s) were funded in FY 1982, giving a total of 30 active training programs supported by the Kidney Disease and Urology Program, which provide stipends for 92 postdoctoral and 4 predoctoral trainees. In addition to the 9 active individual National Research Service Awards (F32s), 11 new awards were made, for a total of 20 fellowships supported with FY 1982 funds. Five new Research Career Development Awards were established, bringing the total to 19 and the Clinical Investigator Award Program increased to 12, with the addition of 3 new commitments.

Meetings

The workshop on Modern Techniques in Renal Research, which was held in Boston, April 1-2, 1982, was co-funded by the National Heart, Lung and Blood Institute and the NIADDK. The two-day workshop was designed to develop a greater interest and motivation towards a research career for 45-50 young nephrologists in-training, by exposing them to experienced senior investigators in nephrology, who demonstrated and explained some of the techniques that are in use in their respective laboratories. New methodologies, some unique to renal research and some borrowed from related fields, were demonstrated.

The Third International Congress on Nutrition and Metabolism in Renal Disease, scheduled for September 1-4, 1982, in Marseille, France received partial NIADDK and Fogarty International Center support to help underwrite the costs of invited speakers and young investigators. A major thrust of the meeting focused on an area of recent activity and high interest, including techniques for the prevention of progression of chronic renal failure as well as other topics in the broad area of nutrition in renal disease. Some 250-300 investigators attended the meeting and proceedings will be published as a regular issue of the American Journal of Clinical Nutrition.

In cooperation with the Diabetes Program, the Kidney Disease and Urology Program provided partial support for the symposium - "Diabetes Mellitus and Renal Disease", held in Arlington, Virginia, October 15-16, 1982. The meeting was organized by the National Kidney Foundation, with co-sponsorship of the American Diabetes Association. Cross-disciplinary in scope, the meeting provided a forum to exchange information concerning diabetes and its effects on the kidney.

The Renal Physiology/Pathophysiology Program of the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases continues to support research on the structure and function of the kidney, such as metabolism, transport and fluid-electrolyte dynamics; diseases and mechanisms leading to chronic renal disease, including diabetic nephropathy, polycystic disease, etc.; specific immune mechanisms of kidney disease, including glomerulonephritis, interstitial and lupus nephritis; and effects of drugs, nephrotoxins and environmental toxins on the kidney. The following are highlights of some of the research activities supported by the Renal Physiology/Pathophysiology Program.

Acute Renal Failure and Organ Preservation

Normothermic ischemic damage to kidneys is a common result of vascular or urologic surgery, especially when cadaver kidneys are harvested for transplantation. According to the extent of this injury, temporary or irreversible acute renal failure necessitating prolonged maintenance on hemodialysis units may result. Three possible mechanisms have been proposed to account for acute renal failure associated with ischemic insult. These are: 1) passive backflow of filtrate across damaged uriniferous tubular epithelium, 2) alterations in renal hemodynamics, and 3) tubular obstruction due to intratubular accumulation of debris. Although some controversy still exists as to the roles of these mechanisms, most evidence indicates that tubular obstruction is probably the primary site of ischemic injury leading to acute renal failure. Histological and physiological studies have also revealed that the major site of this tubular injury and blockage resides at the level of the pars recta.

Of the variety of agents which have been reported to provide some measure of protection from normothermic ischemic injury, mannitol appears to be the most effective. In a recent series of studies undertaken in the laboratory of Dr. Peter M. Andrews of Georgetown University it was found that mannitol (or dextrose when it is used in place of mannitol) is the essential ingredient in clinical preservation solutions which protects the kidney from ischemic damage during prolonged cold storage prior to transplantation. Andrews and colleagues also found, however, that sucrose is even more effective than mannitol in its ability to protect the kidneys from cold ischemic insult during hypothermic storage. Unlike mannitol or dextrose, they found that sucrose is able to effectively preserve (i.e., prevent damage to) the very sensitive pars recta. These observations with cold preserved kidneys led this group to undertake studies to test the effectiveness of sucrose and of some other compounds in their ability to protect the kidneys from normothermic ischemic damage. Preliminary results of these experiments clearly demonstrated that sucrose and some other agents (i.e., very low molecular weight dextrans) can provide the kidney with more protection from tubular necrosis than equivalent dosages of mannitol. Future proposed investigations should provide useful information regarding the ability of sucrose and other osmotic agents to protect the kidney from different degrees of warm ischemic damage.

Renal Pharmacology

In the study of renal pharmacology, plasma membrane vesicles can be used to directly study the membrane transport of pharmacological substrates. Also, drugs and diuretics have been used to define and separate the various transport systems in the kidney. One of the major emphases in the study of kidney function in the laboratory of Dr. Rolf Kinne at Albert Einstein College of Medicine has been the investigation and definition of the membrane transport processes of the individual tubular segments. To study the membrane transport processes in a direct manner, plasma membrane vesicles prepared from the luminal and basal-lateral poles of specific kidney tubule cells, such as proximal tubules, are currently being used. The transcellular membrane transport reactions of such compounds as D-glucose have been defined independent of metabolism, intracellular binding or compartmentalization. By studying a transport system in a purified membrane vesical preparation a better understanding of the function, limits and regulation of the tranport system in the intact kidney can be achieved. In the study of renal pharmacology, drugs and diuretics integrally are being used to define and to separate the various transport systems in the kidney. Conversely, the mode of action of many drugs only can be understood when the specific transport systems upon which they act have been determined.

Lithium Effects on the Kidney

Renal toxicity of lithium salts has been recognized for many years. Because of the increased use of lithium salts for the treatment of neuropsychiatric disorders and their recent implication as a cause of tubulo-interstitial renal disease, there has been renewed interest in the nature of the tubule toxicity induced by this alkali salt. Colleagues of Dr. R. T. McCluskey at the Massachusetts General Hospital have initiated experiments designed to establish the site of the early pathologic lesion and to determine its correlation with the lithium-induced concentrating defect. Treated rats developed marked polyuria accompanied by an elevation of free water clearance and vasopressin resistant diabetes insipidus. Early morphological changes (dilated tubules and cells with enlarged nuclei) were confined to the cortical collecting tubules and possibly contiguous portions of distal tubules. The proliferative component of the lesion was demonstrated by a significant increase in ^{3}H-thymidine uptake by nuclei of collecting/distal tubules. The lesion, evident at three weeks, and including the increased thymidine uptake, extend into the medullary collecting ducts at 18 weeks. Although occasional intratubular mononuclear cells were seen at 9 and 18 weeks, no interstitial inflammation or fibrosis was noted. These lesions are not seen in the kidneys of Brattleboro rats or glucose-treated Wistar rats, which had comparable polyuria. It is suggested that this early and reproducible lesion, characterized by reactive and proliferating tubular cells in the cortical collecting tubules, may predispose the kidney to injury from otherwise mild or insignificant nephrotoxins and may explain the sporadic occurrence of serious tubulo-interstitial disease in patients on long term lithium therapy. A group at the Yale University School of Medicine has also reported that lithium polyuria produces cytoskeletal changes of the principal cells not only of the cortical but of the medullary collecting duct. In an extension of these studies, they have also shown that the cytoskeletal changes are accompanied by alterations of cell volume regulation.

Clinical Trials

Two multicenter clinical trials currently are supported by the Renal Physiology/ Pathophysiology Program. One with its Clinical Coordinating Center located at the Rush-Presbyterian-St. Lukes Medical Center in Chicago is evaluating the effects of plasmapheresis, a regimen widely used to treat a number of "immunologically mediated" diseases, on patients with severe lupus nephritis. The study, now in its second year, will enter 200 patients over a three year period and will include a five year patient follow-up period. An independent NIH Advisory Board/Patient Monitoring Committee, will continue to meet twice yearly to monitor progress and ensure the scientific validity of the study.

The second active clinical trial, comprised of 17 collaborating institutions, has its clinical coordinating center at the Massachusetts General Hospital in Boston. The group is studying a number of adult glomerular diseases, in order to correlate clinical manifestation, course and response to therapy to specific pathological changes in kidney tissue and to ascertain any measurable statistically significant improvement in mortality and morbidity rates conferred by currently proposed therapeutic agents. These studies include: a) adult idiopathic nephrotic syndrome and its response to alternate day prednisone therapy, b) membranous nephropathy including a randomized prospective study of plasmapheresis in treatment of rapidly progressive glomerulonephritis and d) glomerular diseases with non-nephrotic proteinuria. This study also has an Advisory Board/Patient Monitoring Committee, primarily to monitor patient recruitment, data management and the statistical aspects of the study.

A third trial, which began to receive NIADDK funding in late FY 1982, will continue to evaluate the efficacy of cysteamine in the treatment of nephropathic cystinosis, a rare inherited metabolic disorder, characterized clinically by progressive impairment of both renal tubular and glomerular function leading to death, usually before ten years of age. The major biochemical finding is the storage of large amounts of cystine in lysosomes in various organs. The underlying defect has not been defined. Originally begun by 3 investigators in 1979, one at the UCSD, another at the NIH and a third at the University of Michigan, the study now has 53 patients enrolled and 22 collaborating investigators. The study is non-blinded, because of the difficulties in disguising cysteamine and of the need to discuss the patient's leukocyte cysteamine levels so as to regulate the dosage.

Volume Receptors

The role of cardiopulmonary receptors and baroreceptors in modulating salt and water excretion in the nonhuman primate during hypervolemia has not been well defined. The few studies which have been performed in the monkey have however, indicated that blood volume control in this species may utilize different afferent neural pathways than those previously shown to be of major importance in the dog.

Interesting studies underway at the Texas A & M University College of Medicine are addressing the role of cardiopulmonary receptors, baroreceptors, and renal nerves in modulating salt and water excretion during hypervolemia in the monkey. These studies also address the question of head-out immersion as a valid alternative method to outright volume expansion for assessing blood volume homeostatic mechanisms.

This laboratory has demonstrated that cardiac denervation or vagotomy-sinoaortic denervation do not attenuate the renal responses of the monkey to volume expansion. Preliminary data from monkeys that have undergone chronic bilateral renal denervation and volume expansion (15% of blood volume) show that the renal denervated non-primate may have a potent natriuresis following volume expansion. These data would appear to support the idea that the renal nerves do not allow the natriuretic response to be exaggerated, which are markedly different than the reported effects in the dog. The continued generation of data by this laboratory should lead to more clearly delineated roles of the various receptor areas in the volume expanded state of non-human primates.

Renin

A former Clinical Investigator Awardee at the University of Southern California was recently awarded a research grant and an RCDA to continue her studies aimed at characterizing active and inactive forms of renin in normal subjects, pregnant women and patients with diabetes mellitus. Already she has prepared and has begun to characterize semipurified inactive renin from kidney and plasma. By dietary sodium manipulation and changes in posture, she will attempt to support her hypothesis that there may be a defect in conversion of inactive to active renin in diabetes mellitus patients. In addition, she anticipates identifying differences in the biochemical characteristics of renin during pregnancy and thus be able to determine whether or not the rapid increase in inactive renin that occurs during the onset of pregnancy came from the kidney or from the utero-placental unit. The studies in diabetics may reveal a defect or a difference in the conversion of inactive to active renin in this disease state.

Kidney Prostaglandin Synthesis in Tissue Culture

The kidney is an important source for the production of prostaglandins, fatty acid derivatives with a variety of hormone-like actions. The role of renal prostaglandins in modulating renal function in normal and in diseased states has not been delineated. A group of researchers is studying prostaglandin production in glomerular cells grown in tissue culture. A particular cell type that appears after about 2 to 3 weeks in culture resembles mesangial cells (cells from the membrane that supports the glomerulus) in its growth characteristics and in its light- and electron-microscopic appearance. Through use of the prostaglandin precursor ^{14}C arachidonic acid in the medium containing these cells, it has been found that the cells incorporate arachidonic acid and metabolize it to produce prostaglandins. This ability of isolated cells to utilize ^{14}C arachidonic acid to produce prostaglandins was inhibited by the drugs indomethacin and meclofenemate, classic inhibitors of prostaglandin synthesis in other tissues. By isolating prostaglandin production in a specific segment of the nephron, scientists can begin to investigate the role of prostaglandins in the regulation of kidney function in health and disease.

The Urology Program of the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, supports research into the basic mechanisms, pathophysiology and the therapeutic approaches to urologic disorders. These include: benign prostatic hyperplasia (BPH), urinary tract infection (UTI), urolithiasis, obstructive uropathy, neuromuscular dysfunction of the urinary tract, vesicoureteral and renal reflux, and sundry conditions such as Peyronie's disease, impotence, congenital anomalies of the lower urinary tract, etc. Progress in research in a number of these areas is reported below.

Benign Prostatic Hyperplasia

Development of benign prostatic hyperplasia (BPH) is a common occurrence in men, representing a major cause of morbidity over the age of 40. Recently there has been an increase in knowledge about factors influencing the development of BPH, primarily from studies in dogs. Little progress has been made to increasing knowledge in man.

At the University of Colorado in Boulder and Denver, Drs. L. Chung and G. Cunha independently, developed methods for separating stroma from epithelia in rat prostate. Studies of testosterone in these cell populations reveal that dihydrotestosterone (DHT) formation is 5-fold higher in the epithelium of the dorsolateral prostate lobe than in that of ventral lobe. Efforts to correlate morphologic changes during the development of the prostate and the biochemical characteristics of this gland, with hormone cell membrane receptors continue. The experimental data indicate that the amount of prostatic epithelial growth is proportional to the amount of mesemchyma with which it is associated. This significant finding suggests that human BPH may result from expression of embryonic growth potential by stromal components in the gland.

At Johns Hopkins University, Dr. D. Coffey and collaborators recently completed canine cross-sectional aging studies. As the canine prostate ages, it develops an increased sensitivity to androgens which results in enhanced growth per given amount of serum testosterone. This correlates with increase DNA accumulation and number of prostate epithelial cells. It appears that there is a concomitant decrease in the prostate epithelial cell function with resultant decreased ejaculatory volumes, and that there is a 25-30% decrease in serum testosterone while estradiol levels are maintained constant.

Using a flow diagram based on the flux of steroids through the various metabolic pathways an enzymatic-metabolic index for the normal dog was developed. A linear correlation was established between the level of this index and the size of the canine prostate in BPH. It appears that estrogen increases this index with subsequent elevation of tissue DHT and induction of BPH. This discovery points to the important concept that there may be a slow shift in the metabolic pattern and as the increases occur, there is an increase in prostatic hypertrophy. If a similar change in human BPH is confirmed definitively, this will be a major contribution to our understanding of one of the BPH associated biochemical events in both humans and dogs.

Work by Dr. S. Liao and associates (University of Chicago) has increased understanding of ways in which androgens interact with intracellular receptor proteins and their association with cell nuclei chromatin. They are involved with purification and characterization of androgen-dependent proteins; preparation of antibodies against these proteins and to examination of the immunocytochemical localization of these proteins.

Using the dog as the animal model Dr. J. Grayhack and collaborators (Northwestern University) are evaluating the effect of testicular radiation in development and establishment of BPH. A definite effect following testicular radiation has not been observed yet.

Dr. O. Hechter and associates at Northwestern University, in collaboration with Dr. P. Robel and associates, Bicetre, France are assessing factors which may influence the degree of cell damage produced by physical methods of tissue mincing. Results indicate that a large fraction of epithelial cells are "directly" damaged by tissue mincing. Cytotoxic factors are released which secondarily lead to cell lysis and nuclear breakdown.

Dr. R. Lawson and collaborators at the Medical College of Wisconsin, are purifying and characterizing a growth factor -- or peptide hormone -- found in prostatic tissue from patients with BPH.

Urolithiasis

Specialized Centers of Research in Urolithiasis (SCOR's)

Research supported by the congressionally mandated SCOR's begun in Urolothiasis have resulted in understanding of the basic mechanisms leading to stone formation, stone dissolution, and, to possible prevention of the development of kidney stones. The five SCOR's initiated in 1977 are on their final year of support. The productivity of these centers is evident in the number of investigators, publications, steady advances in knowledge, and progress in prevention of recurrences of urolithiasis in a large number of patients.

At Michael Reese Hospital, Dr. F. Coe and associates continue to investigate the pathogenesis of hypercalciuria and calcium oxalate stones. Urine saturation and crystal growth inhibition are measured in stone formers, under various forms of treatment. The effects of urothelial injury on the deposition and attachment of calcium oxalate crystals is also under investigation.

Clinical data on 850 stone forming patients is being analyzed to identify factors influencing the risk of calculi recurrence. Hypercalciuria, an important risk factor was found in more than 40% of these patients. In another study, glycosamonoglycans, acid glycoproteins, RNA and RNA fragments, all normally present in urine, have been shown to be potent inhibitors of calcium oxalate crystal growth.

Dr. B. Finlayson and colleagues at the University of Florida are clarifying the physicochemical features of calcium oxalate precipitation and aggregation, and quantifying urinary crystallization activity. The critical inducing factors necessary to convert a non-stone to a stone-former are being sought. Efforts to characterize the effect of urinary tract inhibitors of crystal growth i.e., citrate, magnesium, pyrophosphate and others in different combinations and the effects of macro and of micromolecules upon the solubility of inhibitors and promoters of crystalization are continuing.

Dr. C. Pak and associates at the University of Texas developed a reliable ambulatory evaluation protocol. This simply designed outpatient work-up for stone-forming patients is being evaluated. The underlying mechanisms leading to kidney stone formation have been determined in 95% of patients studied and long-term hospital admissions to conduct mineral balance studies, are found unnecessary in most patients.

By recognizing the mechanisms that lead to kidney stone disease in individual patients, these investigators are evaluating "optimum" treatment modalities for safety and efficacy, to arrest or to treat identified abnormalities. A significant clinical improvement and remission or reduction of stone formation as compared with the pretreatment programs has been observed.

At Yale University under Dr. H. Rasmussen and collaborators research efforts continue to delineate the pathophysiology of nephrolithiasis and hypercalciuria; the role of 1,25 dihydroxyvitamin D_3 (1,25 $(OH)_2D_3$) at cellular and molecular sites and, the relationship between divalent ions transport across intestinal and renal epithelial. These investigators have demonstrated the likelihood that 1,25$(OH)_2D_3$ regulates calcium transport across the brush border membrane by a primary effect on membrane lipid structure; that there is a close correlation between plasma 1,25$(OH)_2D_3$ concentration and urinary calcium excretion; that patients with primary hyperparathyroidism and recurrent renal calculi have a bihormonal disease with increases in both plasma parathyroid hormone (PTH) and 1,25$(OH)_2D_3$ levels and that most patients with absorptive hypercalciuria have high plasma levels of 1,25$(OH)_2D_3$.

At the Mayo Clinic, Dr. L. Smith and collaborators continue to examine factors that promote and prevent crystal growth formation; the sites of reabsorption and secretion of the major ions within the nephron; the relative roles of supersaturation and inhibitor activity in animal models and patients with calcium urolithiasis; the effects of therapy and the epidemiology and genetics of urolithiasis in a well defined population base.

Prospective clinical studies to reach completion soon, indicate that general disturbances of intestinal absorption in patients with enteric hyperoxaluria may play a role in the formation of renal calculi; that significant differences from normal are observed in the diet composition of idiopathic calcium lithiasis patients; that associated metabolic abnormalities were present in 62% of patients (79) with infected renal lithiasis and, that with a combined medical and surgical approach that includes a metabolic work up, treatment of infection and a long-term follow-up, cure is possible in the majority of these patients.

Analytical Methodology for Measuring Oxalate in Urine

The lack of a simple reliable method of measuring oxalate in biological fluids has been a major deficiency for the study of oxalate metabolism, especially because oxalate is a major component in two-thirds of the kidney stones. Methodologies for oxalate measurement are in the final stages of development, through two contracts and one grant supported by the Urology Program.

Dr. M. Menon, at Washington University is testing, a conductivity chromatography method for oxalate determination.

Dr. J. Peters, of Stanford Research Institute, has developed a reliable and useful clinical laboratory method for measurement of urinary oxalic acid. It is based upon reaction of whole urine with o-phenylenediamine to derivate oxalic acid which is separated and the product measured quantatively by automated liquid chromatography.

Dr. H. Moye, at the University of Florida, has developed a reliable and diagnostically useful procedure for routine clinical laboratory measurement of urine oxalate by a microanalytical gas chromatography technique.

Vesicoureteral Reflux

In a effort to resolve the controversies about management of children with vesicoureteral reflux (VUR), a multi-center prospective study was begun and is in its third year of operation with Dr. S. Levitt, Albert Einstein College of Medicine, as principal investigator. The aim of this study is to determine whether corrective surgery or prophylactic medical therapy of urinary tract infection represents the best approach to the prevention of renal damage associated with VUR. Random allocation of patients with VUR to either medical or surgical therapy, will permit to compare the effect of each of these therapeutic modalities. To generate statistically significant volume of data in a reasonable time frame, collaboration between referral centers (18) in the USA and a similar European study may be indicated.

Fundamental research proceeds at Yale University under Drs. C. Hodson and V. Andriole independently, to define the basic mechanisms by which VUR produces renal fibrosis; to delineate the mechanisms of hypertension developing in reflux nephropathy; to define the role of Tamm-Horsfall protein and the nature of the immune responses observed in reflux nephropathy.

Urinary Tract Infections

Urinary Tract Infections (UTI), are the second most common bacterial disease seen in clinical practice. The goals of prevention of UTI are prevention of renal damage and prevention of recurrent infection.

Dr. J. Roberts and collaborators (Tulane University) continue their investigation of changes in the urinary tract following bacterial infection. They are correlating changes in physiology with studies of humoral and cellular immunity.

Dr. G. Hemstreet (University of Mississippi) is studying bacterial antigens which persist in rats, mice and human kidneys after naturally occurring or artifically induced pyelonephritis. He is assessing whether antibodies, cellular effectors, or both are cytotoxic to cultured kidney cells and determining whether induced "neoantigens" trigger continuing immunological destruction of the kidney.

Dr. D. Uehling (University of Wisonsin) is evaluating the safety and efficacy of vaginal immunization to prevent UTI.

CHRONIC RENAL DISEASE PROGRAM

Introduction

The Chronic Renal Disease Program supports research into pathophysiology of renal failure, renal transplantation and maintenance therapies for end stage renal disease, including hemodialysis, peritoneal dialysis and complications of chronic renal failure

Developments in Kidney Transplantation and Artificial Kidneys

The main techniques used in treatment of chronic renal failure for more than twenty years have been hemodialysis and peritoneal dialysis. In 1981 dialysis was provided for about 56,000 patients under the Medicare End-Stage Renal Disease (ESRD) Program; 4700 transplants were performed during the same period of time, at a cost to the federal government of over $1.3 billion.

The NIADDK supports research on renal transplantation, organ preservation, transplant immunology and immunosuppressive therapy. Transplantation is an effective means of treatment for patients with ESRD when well-matched related donor kidneys are available. It is less effective when cadaver kidneys are transplanted. The requirement for long-term immunosuppression to minimize potential renal graft rejection and high incidence of graft failure has resulted in less consistent therapeutic success in transplant patients than that achieved by ESRD treatment with dialysis. The problem of graft rejection continues to be one of the most serious obstacles in achieving successful kidney transplant. Patients who receive well-matched kidneys from closely related donors have the best chance of success of their renal transplants.

Chronic renal failure and its accompanying "uremia" is a multi-organ system disorder. Symptoms arise during the course of and secondary to dialysis treatment and, also regardless of treatment, in relation to the uremic state *per se*. Examples of these complications include: anemia, renal osteodystrophy, increased susceptibility to infection, central and peripheral nervous system symptoms, cardiovascular complications, sexual dysfunction, endocrine abnormalities etc. In addition, patients on hemodialysis often experience muscle cramps, hypotension and post-dialysis fatigue, especially when large amounts of fluids are removed during dialysis treatment. The mortality rate among maintenance dialysis patients is about nine to twelve percent a year. This figure must be viewed in contrast with the 100 percent mortality for patients with non-functional kidneys if dialysis or transplantation were not available for treatment.

Pathophysiology of End-Stage Kidney Disease

In studies designed to define the pathophysiology of chronic renal disease and uremia, Dr. S. Klahr and collaborators at Washington University, have characterized the adenylate cyclase of human parathyroid hormone (PTH). They have demonstrated that calcium has a profound PTH inhibitory effect in the parathyroid glands at much lower concentrations than in other tissues and that the kinetics of this enzyme are altered in hyper-functioning human parathyroid glands. The effects of 1,25-dihydroxy cholecalciferol ($1,25\ (OH)_2D_3$) on PTH secretion was assessed, to see if $1,25(OH)_2D_3$, independent of its effects on serum calcium, may influence PTH secretion, directly. No evidence for such an effect was found.

Examination of the effect of acute acidosis on the uptake of PTH and cyclic AMP production by isolated perfused bone showed that metabolic acidosis caused both an increase in the extraction of PTH and in the release of cyclic AMP. Changes in CO_2 per se did not lead to changes, which suggest that acute metabolic acidosis may increase bone reabsorption by increasing the uptake of PTH and cyclic AMP production by the skeleton. The role of the liver and kidney in the metabolism of intact PTH was studied with new methods. Results indicate that liver and kidney account for all of the degradation of intact PTH while bone only extracts the 1-34 fragments. A reduction in renal mass, markedly reduces the peripheral metabolism of PTH both in the kidney and liver.

Dr. S. Massry and associates, at the University of Southern California found myocardiopathy related to excess PTH in uremia which is mediated by significantly enhanced myocardial calcium influx and uptake, reduced mitochondrial oxygen consumption and impaired energy transport and utilization as shown by levels of myocardial ATP and creatinine phosphate.

Preliminary data by Dr. R. DeFronzo and collaborators at Yale University indicates that in uremic subjects, insulin-mediated glucose metabolism is reduced markedly as compared with normal controls. Basal hepatic glucose production is normal and is suppressed normally following insulin administration. They postulate that tissue insensitivity to insulin is probably the primary cause of insulin resistance in uremia.

Preliminary experimental data obtained by Dr. D. Emmanouel and collaborators at the University of Chicago, shows that the liver and kidney account for more than two-thirds of the overall degradation of insulin, glucagon and somatostatin in canines. It is interesting to note that the mode of handling of these peptides by both organs is strikingly similar but, the kidney also is involved in filtration, hormone uptake and inactivation of peptides from postglomerular blood.

Dr. Y. Alevy and collaborators, at St. Louis University has developed an experimental model for study of the effects of chronic uremia on the various parameters of immunoregulation. They found that lymphocytopenia is significantly greater at all intervals after induction of uremia than in control animals. They also found that the response to T cell mitogens of spleen cells, peritoneal and alveolar macrophages from chronically uremic rats was significantly more suppressive than was that of the corresponding control cells. They also noted that uremic macrophages are defective in the antigen presentation process to control immune T cells and that uremic T cells do not proliferate in response to antigen presented on control macrophages. The defect apparently lies both in the responding T cell and in the antigen presenting macrophage.

At the University of Colorado, Dr. A. C. Alfrey and collaborators identified dietary phosphorus restriction as an extremely effective means of preventing further deterioration of kidney function in experimental glomerulonephritis and in the nephrotic syndrome. Preliminary data suggests that neither PTH nor renal parenchymal calcification are responsible for the initiation of progressive renal failure, although it appears that these factors gain importance later in the course of renal failure.

At the University of Miami, Dr. J. Bourgoignie and associates working with experimental animal models of chronic renal insufficiency developed the concept that there is limited ability to handle an acute load of sodium, potassium or phosphorus with resultant prolonged abnormalities of electrolyte and water homeostasis. This limitation may be reversed later, but it is further prolonged as renal deterioration progresses. Clinical experience indicates this concept may also be applicable to human subjects.

Renal Transplantation

Dr. J. S. Najarian and associates, at the University of Minnesota continue to maintain the transplant computer data file which provides information on all aspects of kidney transplantation including outcome and longitudinal follow up of patients transplanted at the University of Minnesota since 1968. Information collected includes demographic data, disease status (primary and other), duration and type of dialysis, transplant type, tissue typing information and immunosuppresive regimen. This data base has been helpful in objective documentation of historical changes and clinical impressions.

In another study, these investigators are assessing the value of splenectomy in renal transplant patients. Over a two year period 299 renal allograft patients were randomized into two groups: splenectomized and non-splenectomized; and than stratified according to transplant donor type. The immunosuppressive therapy was the same in both groups: antilymphocitic globulin (ALG) in the immediate post transplant period; and azathioprine and prednisone for the duration of graft function. Patient survival was 85% at 2 years and there were no differences between the groups. Overall, the graft survival rate was 14% better in the splenectomized group. At two years the graft survival rate was 28% better in the splenectomized recipients of cadaver kidneys than in the nonsplenectomized patients. There were no differences in graft survival between treatment for recipients of HLA identical sibling kidney. These investigators concluded that with this immunosuppressive regimen, splenectomy should be done in recipients of kidneys from either cadaver donors or from HLA non-identical selected donors.

In a randomized double blind study, the effectiveness of ALG for reversal of established first rejection in recipients of first renal allografts was established. Patient survival at 14 months was 89% for the ALG treated group and 88% in the placebo group (i.v.human IgG). Graft survival was better with ALG (82%) than with placebo (61%).

A new immunosuppressive drug -- Cyclosporin A (CSA) -- which may have a better therapeutic ratio than the conventional immunosuppressive drugs in general use is under investigation. A randomized prospective clinical trial of CSA in comparison to Azathioprine-ALG is underway following a pilot study. These investigators report that CSA in the pilot study appears to have marked effective immunosuppression for clinical transplantation. However, blood level analysis is necessary for individualization of treatment.

To determine the safety and efficacy of total lymphoid irradiation (TLI) as compared to conventional immunosuppressive modalities in prevention of cadaver renal allograft rejection, Dr. S. Strober and associates at Stanford University are investigating the cellular basis of long-term immunosuppression and tolerance of TLI in laboratory animals and patients. Changes in function and cell surface markers of T lymphocyte subpopulations (induced by TLI) using monoclonal antibodies and fluorescence activated cell sorters are assessed.

Dr. D. Sutherland and collaborators, (University of Minnesota) evaluating TLI to improve renal allograft survival have demonstrated significant improvement in dogs pre-treated with fractionated TLI administered prior to transplantation (150 rads/day, total 2100 rads) plus azathioprine (2.5 mg/kg/day). The efficacy of lower doses of TLI (150 rads/day, total 1800) plus azathioprine (1.5 mg/kg/day) were also tested. Preliminary results indicate that although survival is not significantly different between groups, fewer non-renal related deaths were observed in the second group. Post transplant TLI used with antithymocyte globulin (ATG) was not better than ATG alone. Studies are ongoing now with TLI and either azathioprine or CSA but no data are yet available.

Dr. R. J. Corry and associates (University of Iowa) are continuing their assessment of the role of sharing major or minor histocompatability antigens between blood and organ donor as necessary variables to achieve beneficial effects of blood transfusion in improvement of allograft survival. Preliminary data indicates that mechanisms other than immunological enhancement, may be responsible for the improved allograft survival accompanying prior blood transfusion. Suppressor cells have been implicated in the induction and maintenance of the immune irresponsiveness produced by transfusion. A further understanding of this mechanism will provide clinicians with knowledge of the phenomenon so that transfusion protocols for dialysis patients awaiting transplantation may be developed.

Dr. F. O. Belzer and collaborators, (University of Wisconsin) have conducted studies to attain successful long-term preservation of kidneys. Their data suggests that preservation damage may be a direct consequence of hypothermia on the cellular membrane systems. This damage may cause a disequilibrium of lipid metabolism, increased activity of phospholipases, generation of toxic levels of free fatty acids and the loss of the cellular lipid material, necessary for membrane activity. This damage may result in increased membrane permeability and decreased fluidity. Suppression of these metabolic events appear critical to successful long-term preservation. A new and promising synthetic perfusate containing gluconate as the primary cationic species has been developed.

The Hematology Program of the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases supports investigation into basic mechanisms of normal blood cell function and into pathogenesis of hematologic disorders, and includes development of treatment and prevention modalities, clinical application and evaluation of treatment. This research increases both fundamental and applied knowledge about blood. In addition, the availability of blood and blood cells facilitates study of membrane phenomena, protein structure and function, and cellular differentiation, to obtain information broadly applicable to medical problems such as end stage renal disease, organ and tissue transplantation, arthritis, nutritional disorders, and malignancy.

The Hematology Program, as described above, has been in existence since the formation of the Institute more than 30 years ago. It has always had major emphasis at the cutting edge of basic research, applied across multidisciplinary areas of hematology. The evolution of research has led to a striking emphasis on hemoglobin genetics and hemopoiesis, two initially distinct areas which are becoming contiguous. The Program relates closely to most of the other programs of the NIADDK. Anemias caused by nutritional deficiency and absorption malfunctions are of great concern and are intimately involved with programs in Digestive Diseases and Nutrition. Studies of congenital or hereditary anemias, generally resulting from some specific fixed disorder in body chemistry and molecular design, such as the inherited hemoglobinopathies, red blood cell enzyme deficiencies, and the porphyrias, complement the effort of the Metabolic Diseases Program. There is emphasis on erythropoietin production by the kidney, and the possible therapeutic role of erythropoietin in reducing the severity of the anemia of end stage renal disease. In addition, basic research in HLA and transplantation also relates to important goals of the Kidney Programs. Anemias acquired secondary to arthritis, to endocrine deficiencies, and to other chronic diseases are within the Hematology Program mission.

Red Blood Cell Disorders

Investigators in this field have defined, for the first time, some of the molecular interactions between spectrin and other components of the red cell membrane skeleton in normal and abnormal red cells. Reduced amounts of one specific protein, known as "band 4.1" protein, have been found in patients with a form of anemia caused by red cell fragmentation, called homozygous hereditary elliptocytosis. This finding, by NIADDK-supported investigators, is of major clinical significance, since it is the first demonstration of the clinical consequences of a human membrane skeletal defect. The technique, ektacytometry, has been developed by these investigators to analyse separately the major factors responsible for cell deformability. It has opened up a new avenue of research into the study of cell membrane disorders, which will have a significant clinical impact.

Hematopoiesis

Understanding the mechanisms underlying function of the hematopoietic system is of utmost importance to treatment and possible cure of malignant disorders of the blood forming organs, such as leukemia, multiple myeloma, and aplastic anemia. The hematopoietic system provides a model for the study of cell proliferation and differentiation, since hematopoietic progenitor cells are readily obtainable and may be cloned *in vitro*. Specific proteins are known which regulate proliferation and differentiation, and cell lineages for each differentiation process have been demonstrated. It is possible to study systematically the changes and roles of elements and cellular components of this system in development, differentiation, and maturation. An understanding of these processes will lead to insights into mechanisms of hematopoietic disease, and the differentiation process in general.

It is firmly established that erythropoietin (Ep), produced by the kidney, is the main regulator of erythroid differentiation. Renal injury, resulting in diminished renal mass regardless of etiology, results in a significant reduction in Ep production. Although it is generally accepted that the principal cause of anemia of renal failure is impaired Ep production, the evidence suggests that a suppression of normal erythropoiesis by a hypothetical urinary toxin may be of importance. Another goal of Ep research is the potential benefit of Ep in alleviating the anemia of renal failure, which afflicts virtually all of the 68,000 patients with this disorder.

In a sheep model of renal failure, grantees are studying both the normal and subsequently uremic state to elucidate the effect of uremia *per se* on the responsiveness to Ep. The action of Ep appears to be similar in normal sheep and in those with chronic renal failure. In addition, it appears that the anemia of renal failure, developed in the sheep, can be corrected with daily Ep infusions. Four anemic uremic sheep, receiving daily Ep infusions for 13-40 days, showed complete correction of their anemia.

Hemoglobin

Over the past five years, knowledge of the structure of the genetic regions controlling human and experimental animal hemoglobins has increased remarkably. Recombinant DNA technology, the development of long-term cell culture systems, and other technological advances, have facilitated progress. The complete cluster of human beta-type genes covering over 60 kilobase pairs of DNA has been cloned and each of the five productive genes has been sequenced in normal individuals. In addition, studies of the types of hemoglobin present in single red cells at different stages of development and in the cells produced during *in vitro* culture of single precursor cells of the erythroid series have provided NIADDK-supported investigators with the strong impression that the changeover from fetal to adult globin synthesis is of a clonal nature. Some colonies of cells have the potential to synthesize fetal and adult hemoglobins, and other colonies of cells are able to synthesize only adult hemoglobin, but both are derived from a single precursor. Information on this system will provide important information for use in development of techniques for enhancement of fetal hemoglobin synthesis in patients with defective adult hemoglobins.

Nutritional Anemias and Iron Metabolism

Nutritional deficiency diseases are major medical problems of the world, with an estimated one-third of the world population considered poorly fed. In the United States, deficiencies are common in infants, adolescents, pregnant women, alcoholics and drug addicts, food faddists, and the elderly. Such dietary deficiencies result in anemia. In studies of iron absorption in rats, an NIADDK grantee has found that calcium significantly diminishes the absorption of iron in a dose related manner. Electron microscope studies showed that calcium decreases the entry of iron into the gut microvilli, and delays the transfer of iron from the intestinal absorptive cells into the body. Animals fed a high calcium, iron replete diet developed mild iron deficiency anemia. These studies have significance for human nutrition, particularly in infants. The average American adult daily diet contains a relatively large amount of calcium and quite small amounts of iron. Iron deficiency is common in infants, due to a large iron requirement and the consumption of quantities of cow's milk, which is high in calcium and low in iron. It is strongly suggested that the reason infants are apt to develop iron deficiency when fed on cow's milk rather than human milk is that cow's milk has a higher calcium concentration.

Immunohematology

Transplantation and graft-versus-host disease, as well as numerous immune disorders of the blood and blood forming organs, are supported. In a study of the biological roles of "tissue factor activity" produced by leukocytes in dogs, an NIADDK-supported investigator has found evidence that the process of kidney allograft rejection stimulates mononuclear leukocytes to produce tissue factor. Renal transplantation in dogs which have been bilaterally nephrectomized and given immunosuppression therapy is associated with marked increases of tissue factor production by leukocytes isolated from the circulation. Kidney rejection is also associated with a stimulatory effect on tissue factor production. Rejected kidneys are found to be infiltrated with leukocytes containing high levels of tissue factor activity. It is postulated that the tissue factor activity is involved in the initiation of thrombotic reactions, as the leukocytes respond to injury or inflammation.

Development of Iron Chelating Agents for Clinical Use in Iron Overload

Synthesis and screening of new iron chelating compounds for use in Cooley's anemia patients with iron overload due to chronic transfusion therapy has continued with the addition of subclinical toxicologic evaluations of compounds considered effective in the hypertransfused mouse screen. One new chelating agent will begin toxicologic evaluation during FY 1982, with a second scheduled for early FY 1983. The Program continues to accept and screen compounds from investigators who are not supported by the Institute, as well as from pharmaceutical companies.

Compartmental models of dendritic neurons have been shown to provide a means of computing electric current flow and membrane potential transients that can simulate experiments with injected current or synaptic excitation in real neurons. Although important insights have been obtained with idealized branching models, a significant advantage of compartmental models is that one can match the experimental complexity (with regard both to branching geometry and to membrane nonuniformity) of real neurons as closely as one can specify it. The need to feed such specified complexity into the computer has become more urgent because of recent experimental advances which yield anatomical and electrophysiological data for individual identified neurons. To meet this need, we have devoted considerable time and effort to developing and testing a library of subprograms which convert neuron parameter specifications for complex neurons into input acceptable to two large electrical network modeling programs, SPICE and NET-2, that currently operate on the NIH, IBM-370 computer. This implementation has recently been made available to several colleagues at NIH; it is currently being used to explore several applications to real experimental neurons. A paper is in preparation. (Rall and Miller).

We have explored several degrees of freedom in the parameter space for models of cat spinal motoneurons with dendritic synaptic input. We studied the effect upon EPSP amplitude at the soma for the following: (a) for different dendritic distance to input in all dendritic trees, (b) for similar inputs restricted to one of twelve dendritic trees, (c) for similar inputs restricted to certain branches of one tree, and (d) for doubling and tripling synaptic conductance intensity, to simulate post-tetanic potentiation. Our subprograms constrained certain geometric and membrane parameters and then adjusted neuron size to correspond to input resistance values from 0.5 megohm to 3.5 megohm in steps of 0.5 megohm to match the experimental range. The computed results explicitly verified our intuitive conjecture that the smaller neurons, having the larger input resistance, would not only produce larger EPSP amplitudes, but also (because greater depolarization at the input site must produce a non-linear deficit in synaptic current and in EPSP amplitude) they exhibit reduced factors of post-tetanic potentiation, as observed experimentally. This result not only confirms our physical intuition, but also refutes arguments put forward elsewhere in explanation of such experimental observations. A paper is in preparation. (Rall, Miller, Burke and Lev-tov).

The average electrotonic length of cat spinal motoneuron dendrites is about 1.5 length constants. Why are such dendrites this long, if synaptic input at their tips contribute little to integrative processes at the spike initiation zone? In previous studies, investigators have usually approached this general question by calculating the dependance of synaptic efficacy upon synaptic location. Here, we have taken an alternate approach, and have calculated the dependance of synaptic efficacy upon total dendritic length. Using a compartmental computer model of a simple neuron, we (1) fixed the location of a synaptic contact on one dendrite, and (2) varied the length of dendrite extending beyond the point of synaptic contact. We obtained the somewhat surprising result that the extension or "growth" of the dendrite beyond the point of synaptic contact can increase the efficacy of that synapse, under certain conditions. For small amplitude PSP's, there was no increase. However, for large amplitude synaptic conductance changes (those resulting in local PSP amplitudes that are a substantial fraction of the synaptic driving potential) the optimal dendritic length was found to extend substantially beyond the input location. We speculate whether some dendrites may be "long" in order to increase the efficacy of proximal synapses. The intuitive key to understanding these computed results is that less charge is transferred across the synaptic membrane (for a given synaptic conductance transient) in the case of shorter dendrites, because the larger local depolarization reduces the driving potential for synaptic current. A paper is in preparation. (Miller and Rall).

Combined anatomical and electrophysiological data for individual dendritic neurons have recently been obtained and analyzed by several investigators. Assuming uniform passive membrane properties, the resulting estimate of Rm for each neuron, together with the estimate of the membrane time constant for the same neuron, has usually implied Cm values that are 2 or 3 times the one microfarad per square centimeter value established in the 30's by Cole, Curtis and Fricke. This has led us to explore the implications of assuming a nonuniform membrane where the value of Rm increases with distance from the soma in each dendritic tree, while the value of Cm is held uniform. With both compartmental and continuous neuron models, we find that this complication produces interesting shifts in the mathematical eigenvalues that determine the system time constants. Also, there are interesting questions concerning the interdependence of physiological parameter estimates. Preliminary results suggest that this will make it possible to fit known experimental constraints with Cm values closer to that of Cole. (Rall, Miller and Rinzel).

The analyses of the facilitated transport of oxygen by the carrier hemoglobin or myoglobin have proceeded. The numerical analysis effect of membranes impermeable to the carrier on the diffusion path was completed; the results are contained in a paper published in the Biophysical Journal, May 1982. It was shown that at the higher oxygen concentration side of the membrane the flow of combined oxygen is transferred to the flow of dissolved oxygen. The reverse process occurs at the lower oxygen concentration side. Jump discontinuities on the concentration of the oxygen-carrier compound at the membranes are associated with these transfers. Let Δ_i be absolute value of the jump in the concentration at the i-th membrane. It was shown that the facilitated transport can be represented by

$$F_f = \frac{D_2}{\ell}\,[x_2^{eq}(x_1^0) - x_2^{eq}(x_1^\ell) - \{\Delta_0^+ + \sum_{i=1}^{n} (\Delta_i^- + \Delta_i^+) + \Delta_{n+1}^-\}] ,$$

where D_2 is the diffusion coefficient of the oxygen-carrier, ℓ is the width of the slab, + represent the higher oxygen concentration side of the membrane, - represent the lower concentration side, n is the number of membranes and $x^{eq}(x_1^0)$, $x_2^{eq}(x_1^\ell)$ are the equilibrium concentrations of oxygen-carrier at the faces of the slab. One observes from the expression above that the decrement in facilitation depends on the cummulative effect of the jumps. Let σ_i denote the boundary layer width at the i-th membrane. When the intermembrane distance is larger than $2\sigma_i$, it is possible to show that a relationship exists between Δ^i, the total flow of oxygen, the slope of the oxygen dissociation curve and the chemical kinetics between oxygen and the carrier. These relations define an algebraic system whose solution gives the facilitated flow. This algebraic system enables one to find the facilitated flow directly without having to solve for all the features of the solutions.

Wittenberg (1966) measured the facilitated transport for a number of hemoglobins. He reported that the hemoglobin of the Ascaris body wall does not facilitate the transport of oxygen. This statement has been incorporated in recent reviews (Fairbairn, Von Brand) and papers (Chvapil et al.) on the physiology of the parasites. However, Wittengerg's experiments seem to have been carried out for values of oxygen concentration in the range of 70 to 600 torr. It does not appear prima facie that the results obtained could be extrapolated to the low oxygen concentration of the habitat of the adult parasite in the intestinal lumen of the host. With this in mind, we studied theoretically the behavior of the facilitated flow for values of oxygen concentration in the range 0.5 to 360 torr. It was corroborated that for values larger than 70 torr the facilitation is negligible. However, for values between 2 and 10 torr the facilitated flow has a maximum and it is about five times larger than the flow of oxygen contributed by pure diffusion. Similar results were obtained when the parameters corresponded to the ones of the adult parasite in the intestine of the host. Also a striking parallelism was observed between this behavior and the reported (Fujimoto and Prockoff) activity of the enzyme prolil hydroxilase involved in the synthesis of collagen by the adult Ascaris lumbricoides. Further, a similar agreement was found between the maximum facilitated transport activity for the carrier myoglobin and the average partial pressure of oxygen in striated muscle. (Gonzalez-Fernandez and Atta).

We have started a collaborative effort with Dr. E. Cabib, NIADDK, to study the morphogenesis of the yeast, Saccharomyces cerevisiae. The approach is basically to incorporate the biochemical information that is being obtained experimentally into a mathematical formalism of growth. A model for the growth of the septum between mother and daughter cells and its numerical implementation are being developed. The solution of these equations are to be compared with the experimental information. It is expected that this interaction will cross stimulate experimental and theoretical enquires. (With L. Lara, and R. Mejia). (Gonzalez-Fernandez & Atta).

The Belousov-Zhabotinskii chemical reaction in a flow reactor exhibits bursting patterns of oscillations. Corresponding solution behavior of a simplified three-variable model has been analyzed (Rinzel and Troy, J. Chem. Phys. 76:1775-1789 (1982)). For a certain range of flow rates, there is a net increment in the product P during each cycle. The accumulation of P after several cycles then extinguishes the oscillations. During the ensuing quiescent phase, there is a slow decrease in P and the oscillations resume again when P falls sufficiently. To complement our previous analysis we have formulated and analyzed a one-variable, discrete-time, model for the evolution of P from the beginning of one cycle to the next. The map $P_n \rightarrow P_{n+1}$ exhibits solutions and dependence on flow rate in agreement with those of the three-variable model. These results have been presented at several conferences. (Rinzel and Troy).

When the Belousov-Zhabotinskii system is run without malonic acid $[Br^-]$ slowly decays until it reaches a critical level whereupon the consumption rate explodes and Br^- is rapidly depleted. For the system in a Petri dish, a spatially localized stimulus can prematurely initiate a wave of Br^--consumption which propagates through the medium. By singular perturbation methods, for small $[H^+]$, we have analyzed (J. Phys. Chem., in press) the wave solutions of a two-variable rapid-consumption model. We have determined the propagation speed and its dependence on experimental parameters. (Rinzel and Ermentrout).

To explore the effect of extracellular K^+ accumulation on nerve firing, solutions of the space-clamped, four-variable, Hodgkin-Huxley equations and their dependence on the parameters applied current, I, and extracellular $[K^+]$, C_k were computed. Consistent with experimental results, the model exhibits an N-shaped steady state current-voltage relation for a range of increased C_k values. Hence for a subset of I and C_k values, the model has complex dynamic behavior with multiple steady state potential levels and, in some cases, coexistent stable oscillations. We have also studied numerically the signal propagation characteristics of a bistable Hodgkin-Huxley axon, some of these results parallel, and are motivated by, those found previously for a two-variable FitzHugh-Nagumo model (Rinzel and Terman, SIAM J. Appl. Math., in press). (Rinzel).

Previous work (with W. Rall) on modeling of dendritic spine function and its role in neuronal plasticity was reviewed and presented at the 1982 AAAS Symposium, Some Mathematical Questions in Biology. A manuscript will appear in the Symposium proceedings. (Rinzel).

We have continued our investigation of the qualitative properties of stationary homogeneous neural networks. In a recent survey article we described the behavior of waves and spatial patterns in such nets and the effect of connectivity on these forms. We are able to describe the ragged edges appearing in scintillating scotomas of migranous visual auras. This work will form a chapter in a book on pain to appear shortly. (G. B. Ermentrout)

Buck and Hansen have a large amount of data on the synchronization and response of flashing in East Asian fireflies. The qualitative behavior of these insects is very closely related to the behavior of human circadian clocks. Several simple models are able to quantitatively explain much of their data. A short article on this work is in preparation. (J. Rinzel, J. Buck, F. Hansen, & G. B. Ermentrout)

We continue our investigation of coupled oscillators as models for intestinal slowwaves. A manuscript on the first part of this project (the odd oscillator case) has been prepared and will be submitted shortly. We have focused on the effects of non odd coupling functions, anisotropy and inhomogeniety. The continuum model has been proposed and investigations of this are underway. This work has been presented at numerous conferences. (G. B. Ermentrout and N. Kopell).

We have found that when differential equations admit a certain third order degeneracy, this singularity can be unfolded and a simple third order system appears. It has the form

$$\dddot{x} + \mu\ddot{x} + \nu\dot{x} + \gamma x + x^2 = 0 .$$

We have shown the appearance of chaos in this simple system. It can be derived from a variety of neural systems including a modified FitzHugh-Nagumo equation and the Wilson-Cowan equations for neuronal nets. A manuscript is in preparation. (G. B. Ermentrout)

We have studied phase response curves and periodic forcing of certain neural equations with very long periods. Our results are quite general and can be applied to any system near the transition between excitable and oscillatory behavior. The results are presently in preparation. (G. B. Ermentrout).

The Stein model of stochastic neuronal excitation postulates Poisson distributed incoming signals which are integrated at the nerve cell membrane. Between the impinging impulses passive decay of the membrane potential is modelled. When threshold is exceeded the cell fires and after a refractory period again becomes excitable. The Markov process corresponding to this model has been investigated in the past and methods for calculating the moments of the interspike interval distribution have been developed, though in the case dealing with both, excitatory and inhibitory input this has been difficult and of uncertain accuracy.

In the case of excitatory input only, a method based on power series solution of the Laplace transform of the delay partial differential equation of the system has been developed. This allows very rapid (under 1 sec.) calculation of the interspike interval distribution with excellent accuracy when compared with solutions based on a much more time consuming spatial discretization of the problem. A paper containing these results is in press. In the case when both excitatory and inhibitory input are allowed a numerical method of solution based on a finite state continuous time Markov process has been developed. The average calculation time for an interspike interval distribution is under 1 min. with this method and it has proven accurate when compared with Monte-Carlo simulations of the solution. From this work a number of errors in the literature have come to light. Results are currently in the first draft stage. (Wilbur and Rinzel).

It has been known for some time that if the DNA sequence is considered as the output of a homogeneous Markov process then there are clear differences in level one memory or statistical interdependence of the bases in the sequences from prokaryotic and eukarotic organisms. Using information theoretic measures of the dependence of the third base of each codon on its nearest neighbors and appropriate simulations we have shown that differences in the strategy for choosing the third base are adequate to explain the observations regarding the memory of the complete sequences for prokaryotes and eukaryotes. In particular it is shown that for eukaryotes there must be a context dependence in the choice of codons to represent a given amino acid sequence, i.e., the correct frequency of usage of different codons that represent the same amino acid is not enough. A paper has been submitted and further work is in progress. (Lipman and Wilbur).

Currently the most prominent theory of acoustic pitch perception in the range of verbal stimuli is the place or pattern theory (modified place theory) of pitch perception. Several considerations relating to the phase locking of auditory fibers to periodic stimuli have recently led to the proposal that some form of space or time cross correlation analysis is involved in pitch perception. An attempt is under way to develop a mathematical model of the pattern of auditory nerve fiber stimulation that will allow discrimination between proposed models of pitch perception based on phenomena such as masking, critical bands, missing fundamentals, and other complex tonal aggregates. (Wilbur).

Using two statistical measures derived from Information Theory we have characterized the constraints toward nonuniform base composition and on the ordering of the bases, in a broad range of nucleic acid protein coding sequences. Mitochondria appear quite different from all other coding sequences in this analysis; eukaryotic genes can be divided into 2 groups, those with introns and those without introns, and we have noted a relationship between these statistical measures of a subsequence and its degree of conservation in related genes (Lipman & Maizel, Nuc. Acids Res., 10:2723-2739 (1982)). (Lipman).

We have compared the hemaglutinin genes of influenza in a hierarchical fashion. The comparisons are at the level of homology, doublet frequencies, codon defined position specific doublet frequencies, and the divergence from uniform base composition and independence of the bases. This analysis revealed relationships between subsequences of the genes and between subtypes which would not be evident with a standard search for homology (Lipman, T. Smith, M. Waterman, submitted to Nuc. Acids Res.). (Lipman).

We have analyzed the constraints on synonymous codon choice in prokaryotes, eukaryotes and mitochondria. The degenerate third base in mitochondria shows weak statistical dependence on either of its nearest neighbors. The third base in prokaryotes shows dependence on the second base of the codon and only weak dependence on the first base of the adjacent codon. In eukaryotes, the degenerate third base is dependent on both its nearest neighbors and thus any model for choice of synonymous codon in eukaryotes must involve a constraint on the context of the codon in the sequence (Lipman, Wilbur, submitted to J. Mol. Biol.). (Lipman and Wilbur).

We are characterizing the stationarity of the singlet, doublet, and triplet frequencies in nucleic acid sequences and correlating this property of sequences with biological function. (Lipman and Wilbur).

We have developed a rapid method for the analysis of sequence homology and are implementing it to allow a researcher to automatically compare his query sequence with all the sequences in a data bank. This will allow a researcher to find evolutionary relationships to a query sequence, and will aid in assigning function to newly sequenced DNA. (Lipman and Wilbur).

Work has continued on studies of the mechanism of uninary concentration. Diffusive dissipation, which is an approximation of vascular washout of solute, has been incorporated into the anlytic treatment of the n-stage central core model. The existence of such dissipation seems to be essential for the existence of a stable upper branch of the concentration ratio. We have also extended our analytic treatment of multisolute systems. We have shown that under idealizing assumptions in a system with nephrons with both short and long loops of Henle the maximum concentration ratio that can be generated in the inner medulla by the passive mechanism is

$$x = \frac{1 + n}{1 + n\ C_{1U}(0)/C_{1M}(0)}$$

where n - 1 is the ratio of short to long nephrons and $C_{1U}(0)/C_{1M}(0)$ is the ratio of urea concentration to total concentration in tubular fluid at the end of the proximal tubule. This work is being extended to include the effects of urine withdrawal and vascular dissipation. These both tend to limit concentration as the ratio of short to long nephrons increases. Computer simulation of the system shows an optimum ratio of short to long nephrons of somewhere between 3 and 10. This is consistent with the known architecture of the medulla in desert rodents and the hypothesis that these rodents utilize the passive mechanism for concentration in the inner medulla. (Stephenson).

A project has been initiated on the analysis of the transport of p-aminohippurate and related substances in the mammalian kidney. This project is directed primarily toward the analysis of the kinetics of transport of radionuclide such as 131-I-Hippuron that are used in clinical studies. We have now developed a stable numerical scheme for approximating the differential equation that describes the uptake of PAH by the proximal tubule cells and its subsequent course through the kidney. This scheme and its computational results were presented at the FASEB meetings (Fed. Proc. 41:1240 abstract 5663) and are being written up for publication. The next phase of this project will be to develop procedures for estimating the parameters of the model from images obtained with a scintillation camera following OIH injection. (Kaimal, Kellogg, Stephenson).

Five theoretical principles that follow from qualitative consideration of renal architecture and tubular permeabilities have been proposed to explain the concentration of urine in the mammalian kidney. These are: (1) The medullary loop of the doubly folded S-shaped configuration of the nephron permits solute supplied by ascending Henle's limb (AHL) to extract water from descending Henle's limb (DHL) and collecting duct (CD). (2) The cortical loop allows the diluted

AHL fluid to return to isotonicity with cortical plasma before returning to the medulla. (3) The folded vasa recta and surrounding interstitium (the central core) provide an expansion chamber for the performance of osmotic work and a mixing chamber for salt and urea. This mixing induces passive salt transport out of AHL. (4) Overall, the system acts as a solute cycling multiplier from AHL to vascular core and the osmotically equilibrated DHL and CD. (5) The short-looped nephrons provide urea to drive salt transport out of AHL of long nephrons in the inner medulla. This work was presented as part of a symposium on the urinary concentrating mechanism, organized and chaired by J. L. Stephenson at the Spring, 1982, FASEB meetings. (Stephenson).

A manuscript describing a path-following study of the solution surface of a multinephron kidney model has been prepared for publication. It shows that the transition from the non-concentrating kidney to the concentrating (or diluting) kidney lies on a convoluted solution surface. For a given set of model parameters there may be no solution; the solution may be unique, or there may be a multiplicity of solutions. Experiments done with two nephron populations have shown that the passive concentrating mechanism in the inner medulla is enhanced by urea reabsorbed from the thick ascending limb of Henle via the vasa recta. Recycling via the descending loops of Henle is shown to reduce the ability to concentrate. Conversely, a central core model with two nephron populations shows that urea recycled in the short loops of Henle will enhance concentration.

A path-following procedure for studying solutions to large, sparse systems of nonlinear equations has been implemented and described (Mejia, SIAM Conference on Applied Linear Algebra, 1982). (Mejia).

In quest of efficient solution of models with many nephron populations, we have developed an adaptive multigrid differencing algorithm. The partitioning scheme described previously (Mejia and Stephenson, J. Computational Phys., 1979) is used in conjunction with the finite difference scheme of Tewarson (Math. Biosci., 1981), and a halved mesh size for the global variables in the partitioned scheme. Although this algorithm has the desired accuracy and speed, it allows spurious solutions to the differential equations, thus requiring improvement. (Mejia).

The continuation algorithm, SCOUT, has been used to develop an interactive solver for small systems of partial differential equations. This solver has been used to test various discretization schemes for the analysis of PAH transport in the kidney, with the selection of a backward scheme in both space and time as most appropriate. (Mejia).

SCOUT in conjunction with the symbolic manipulator MACSYMA has been used to solve and do a first order sensitivity analysis for a single-solute 4-tube central core model (Mejia, Navy MACSYMA Users Mini-Symposium, 1982). Without diffusion (as well as for a large diffusion coefficient) the sensitivity analysis shows that multiple (time) stable states do not exist for small variation of model parameters (see above: Garner, Kellogg and Stephenson). For moderate diffusion multiple stable states exist. (Mejia).

Work has continued on the mathematical analysis of a simple model of the renal medulla. The model uses a system of differential equations to represent the flow of water and a single solute in a 4-tube flow network, representing the loop of Henle, the collecting duct, and interstitial space. The model includes active transport out of the ascending limb, water extraction from the descending limb, and passive transport of water and solute to and from the collecting duct. An existence theorem is proved for solutions of the model equations which generalizes a previous result obtained for a 3-tube model of the renal medulla. A simplified system of equations is obtained for the limiting case of large water permeabilities, and the simplified system is solved exactly. It develops that, for a given system of boundary conditions, there may be one, two, or three solutions to the simplified system. The solution surfaces have been explored for a variety of parameter values. (Kellogg and Garner).

A study has been made of the system of ordinary differential equations that describes the flow of water and a solute in a system of parallel flow tubes. The tubes are allowed to exchange water and solute with each other through the tube walls. Several different types of boundary conditions at the ends of the tubes are treated. These boundary conditions are designed to model open-ended tubes, closed-ended tubes, or connections between two tubes of the system. Some *a priori* bounds on the solutions of the system are obtained. These bounds have the consequence that the concentration profiles are positive and that, if there is no active transport of solute in the system, the concentration never exceeds one of the specified inlet concentrations. This latter result generalizes an earlier result of Stephenson (Biophysical J. 6:539-551, 1966). The bounds are used in a fixed point argument to obtain existence theorems for solutions of the nonlinear system. The results have appeared. (Kellogg and Garner).

Work has started on a local existence theorem for a general multisolute model of the full nephron. The goal of the study is to generalize the above results and include the most general flow network that might be used in renal models. (Stephenson).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 13,000-09 MRB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Mathematics of kinetics and reaction-Transport systems

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: John Z. Hearon

COOPERATING UNITS (if any)

LAB/BRANCH
Mathematical Research Branch

SECTION

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is terminated.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 13,001-09 MRB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Mathematical formulations and analysis relevant to experimental neurophysiology.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Wilfrid Rall	Senior Research Physicist	MRB, NIADDK
OTHER:	John Miller	Guest Worker (Postdoc. Fellow)	MRB, NIADDK

COOPERATING UNITS (if any)

Prof. Gordon Shepherd	Dr. Robert E. Burke & Colleagues, LNLC, NINCDS
Yale Univ. Med. School	Drs. P. G. Nelson & P. Guthrie, LDN, NICHD
New Haven, CT	Dr. Barry Bunow, LAS, DCRT

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Mathematical Research Branch

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INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.8	1.5	.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) This research involves creating, exploring and testing mathematical models of dendritic neurons that are relevant to experimental neurophysiology and neuroanatomy. Together these models provide a theory that can account for various sequences of events in the soma and dendritic branches of a single neuron, and for field potentials generated by certain cortical populations of neurons. Computational experiments performed with these models provide theoretical predictions that have been compared with experimental results obtained by colleagues with motoneurons of cat spinal cord, and with the mitral cell and granule cell populations of rabbit olfactory bulb. Resulting interpretations contribute to understanding of dendritic synaptic input and of dendro-dendritic synaptic interactions. Results published in the Biophysical Journal and in the Journal of Neurophysiology are summarized in Chapter 3 of *The Handbook of Physiology: The Nervous System, Vol. I.* American Physiological Soc. (1977) and in a Chapter entitled "Functional Aspects of Neuronal Geometry" in *Neurones Without Impulses*, SEB Seminar Series, Cambridge Univ. Press (1981).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 13,002-10 MRB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Mathematical description of the substrates transport in the capillary-tissue structures.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Jose M. Gonzalez-Fernandez	Research Mathematician	MRB, NIADDK
OTHER:	Susie E. Atta	Mathematician	MRB, NIADDK

COOPERATING UNITS (if any)

Dr. Enrico Cabib	Raymond Mejia	Dr. Lorenzo Lara, Visiting Scholar
LMB, NIADDK	IR, OD, NHLBI	Univ. of Maryland
		College Park, MD

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INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 2.45	PROFESSIONAL: 2.15	OTHER: .3

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

To develop mathematical models of the blood flow and transcapillary exchanges in capillary networks. An effort is being made to incorporate in the models the histological structure of capillary networks as well as different flow patterns from available experimental information. In this model the extraction of substrates with different chemical kinetics at the tissue site will be described. It is expected that this could be used in experimental situations where the extraction of different substrates are measured simultaneously, thus helping to infer the flow pattern features of the microcirculation. In particular a model of the diffusion-consumption of oxygen in striated muscle containing myoglobin (facilitated diffusion) is being developed and pertinent numerical results examined.

A possible adaptive mechanism for the facilitated transport of oxygen by hemoglobin in nematodes (Ascaris lumbricoides) and myoglobin in vertebrate striated muscle has been shown.

PHS-6040

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AM 13,004-08 MRB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Mathematical description of cellular neuroelectric signal transmission.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	John Rinzel	Senior Scientist	MRB, NIADDK
OTHER:	G. Bard Ermentrout	Staff Fellow	MRB, NIADDK
	W. John Wilbur	Medical Fellow	MRB, NIADDK
	Donna Washington	Summer Employee	MRB, NIADDK

COOPERATING UNITS (if any)
W. C. Troy, Assoc. Prof., Math. Dept.
University of Pittsburgh
Pittsburgh, PA

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NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.3	1.9	.4

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project continues to focus on the formulation, analysis, and biophysical interpretation of mathematical models which describe various aspects of neuroelectric signaling for individual neurons. Among the topics of current interest are:
(i) integration of synaptic input delivered to the soma and dendritic branches of a neuron; (ii) steady propagation of action potentials along axons;
(iii) stimulus-response and threshold properties for repetitive-firing of action potentials.

Mathematical models of these phenomena involve systems of linear and nonlinear ordinary differential equations and parabolic partial differential equations. Solutions and their mathematical stability are determined by analytical and numerical methods. One aspect in the approach of this project is to expose the qualitative mathematical structure for classes of models by exploiting simple, yet reasonable, equations. Because qualitatively related mathematical problems arise in other biological and chemical contexts, e.g. chemical and biochemical oscillations, this project may consider models from such applications.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 13,013-03 MRB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Mathematical modeling of biological waves.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. Bard Ermentrout	Staff Fellow	MRB, NIADDK
OTHER:	John Rinzel	Senior Scientist	MRB, NIADDK

COOPERATING UNITS (if any)

Dr. Nancy Kopell, Professor
Northeastern University
Boston, MA

Dr. John Buck
LPB, NIADDK
NIH

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INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 1.8	PROFESSIONAL: 1.3	OTHER: .5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project focuses on the formulation, analysis, and interpretation of mathematical models which describe biological patterns such as waves and oscillations. The main topics of interest are:

(i) neuronal networks and their application to migraines, epilepsy, and phosphenes;
(ii) excitable and oscillatory systems and their behavior under forcing;
(iii) chaotic neural and chemical networks;
(iv) chains of coupled oscillators as models of intestinal waves.

PHS-6040

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 13,014-01 MRB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Probabilistic Analyses of Nucleic Acid Sequences

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	David J. Lipman	Medical Staff Fellow	MRB, NIADDK

COOPERATING UNITS (if any)

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INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 1.04	PROFESSIONAL: 1	OTHER: .04

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project has focussed on the analysis of nucleic acid sequence data as it pertains to molecular biology and molecular evolution. Current areas of interest include:
- i) comparative analysis of mitochondrial, prokaryotic and eukaryotic genes;
- ii) analysis of the constraints on protein coding regions other than the amino acid sequence;
- iii) development of rapid methods for the analysis of sequence homology;
- iv) probabilistic models of nucleic acid secondary structure.

We have employed standard statistical tools as well as statistical measures derived from Information Theory. Because the statistical models become complex and not answerable to analytical solution, Monte Carlo methods are frequently employed.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 13,015-01 MRB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Probabilistic modeling of biological information systems.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W. John Wilbur	Medical Staff Fellow	MRB, NIADDK
OTHER:	John Rinzel	Senior Scientist	MRB, NIADDK
	David J. Lipman	Medical Staff Fellow	MRB, NIADDK

COOPERATING UNITS (if any)

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INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 2.06	PROFESSIONAL: 2	OTHER: .06

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Work is currently under way in three areas involving statistical modelling of biological information systems. The first area deals with the so-called Stein model of neural integration and firing which is in essence a continuous time continuum state Markov process. This process is mathematically described by a first order delay and advance type partial differential equation and numerical methods of solution have been developed for this system. The second area involves an investigation of the statistical interdependence of adjacent bases in mitochondrial, prokaryotic and eukaryotic DNA. It is found that the third base position of the codon has different dependencies for the three mentioned classes of DNA. The third area of probabilistic modelling is aimed at developing a mathematical description of pitch perception based on known models of cochlear mechanics and postulated mechanisms of neural processing in an attempt to give a theory consistent with such phenomena as masking, critical bands, and missing fundamentals. Work is just beginning in this area.

Annual Report of the Laboratory of Cellular and Developmental Biology

During the past year the name of our research group was changed from the Laboratory of Nutrition and Endocrinology to the Laboratory of Cellular and Developmental Biology. While the absence of the initials LNE from the organizational listings of NIADDK may be noted with concern by some, the mission of the group had changed sufficiently that a name that more accurately reflected the types of research underway was needed badly. Major areas of research interest include chromatin structure and gene regulation, development and differentiation, lipid transport, hormone action, enzymology, and hepatic and intestinal metabolism; these are appropriately embraced by the new name of the laboratory.

Chromatin Structure

In several systems both described by others and under study in this laboratory, nucleosome phasing on DNA has been documented for specific genes, that is, histones interact with specific DNA sequences while other DNA sequences are in the linker regions between nucleosomes. One of the most appropriate systems for study of such unique histone-DNA interactions is provided by the ability to "clone chromatin" in yeast, described in our last report. A plasmid containing a TRP1 gene and an ARS1 replicator exists in yeast as a highly amplified episomal minichromosome. Nine nucleosomes are present on the 1453 base pair (bp) circular DNA and interestingly, the positions of the nine histone multimers along the DNA sequence is unique, providing a clear example of rigid phasing.

Another yeast gene, that for the phosphate repressible acid phosphatase (Pho 5), also exhibits nucleosome phasing under certain circumstances. This gene is strictly regulated by the phosphate concentration of the medium; it is active in low Pi and repressed in high Pi. When the gene is active, nucleosome phasing is seen only in regions external to the transcribed sequence and its upstream control regions. In contrast, when cells are grown in high Pi and the gene is inactive, phased nucleosomes are present on the gene sequence also. Other features now known to be characteristic of active genes in higher organisms also occur for the Pho 5 gene in yeast. Thus, hypersensitive sites for cutting by DNAase I near the 5' end of the gene are present when the gene is active and absent when the gene is repressed. Similar observations have been made for the TRP1-ARS1 plasmid under conditions where measurements of mRNA prove the activity of the bulk of the plasmid genes. We have constructed plasmids which contain the Pho 5 gene and other yeast sequences in order to allow us the opportunity to examine in more detail the biochemical mechanisms which lead to phasing and the structural determinants of gene activity or repression.

We have documented one possible mechanism for the occurrence of unique histone DNA interactions. Association of a 260 bp fragment of sea urchin DNA, containing a gene for 5S rRNA, with chicken histones leads to formation of a nucleosome which is precisely positioned, beginning 70 bp to the 5' side of the start site for transcription and terminating 75 bp into the gene region. Thus, in this case, features present in the DNA sequence and histones alone suffice to determine the precision of their interactions.

Since this nucleosome is precisely phased on the DNA and since the cloned DNA sequence can be obtained in high amounts, we have been able to, for the first time, examine the structure of a nucleosome core particle by looking at histone-DNA interactions for the individual DNA strands. End labeling and secondary restriction can generate DNA fragments labeled uniquely at any of the four possible sites for analysis by established methods of mapping accessibility of the DNA in a nucleosome to various nucleases. The results are dramatic in indicating that the environments of the two strands are not identical; not only do histones select a precise location on a DNA segment to which to bind, but they select one strand for one type of interactions and the other for another. These findings seem to require major modifications in the currently accepted model for the structure of this chromosomal element. *In vitro* construction of altered DNA related to this cloned sequence should allow elucidation of the features sufficient for phasing of nucleosomes.

Development and Differentiation

Studies in this area are a major effort in the laboratory and span a range of experimental systems from unicellular eukaryotes to cultured mammalian cells. A new effort this year is study of gene organization and expression in *Dictyostelium discoideum*. This simple eukaryote has a defined short developmental cycle which culminates in production of a spore producing aggregate of two distinct cell types.

We have been studying a short, transcribed sequence (M4) which occurs about 100 times in the *Dictyostelium* genome. We have shown that the M4 sequence is at the 5' end of different single copy - derived messenger RNA's. Expression of both the M4 sequences and several of the mRNA's which are covalently linked to M4 increases more than 5-fold during development, relative to vegetative cells. This suggests that M4 sequences may be important in coordinate control of expression of a number of genes necessary for *Dictyostelium* development. Other developmentally regulated genes are also under study in this organism; one such recently identified gene is repressed rapidly at the initiation of the developmental process. This gene lies within 1500 base pairs of a gene with a totally different pattern of expression during development, suggesting that gross chromosomal alterations are not occurring during this process.

Gene organization has been studied also during development of sea urchin embryos. The histones produced early in sea urchin embryogenesis are distinctive and afford an opportunity to study gene organization prior to activation, during transcription, and after repression. The chromatin structure of these genes shows changes similar to those expected from the results of the Pho 5 gene study in yeast outlined above. Remarkably, even when the genes are maximally active, at morula stage, nucleosome phasing persists in the nontranscribed DNA regions between the histone gene sequences. The extent of cytidine methylation in the sequence CCGG is not altered during developmental activation of the genes. We do not confirm, using more definitive methods, the suggestion made by others that a portion of the histone gene is single stranded DNA during the phase of maximal transcriptional activity.

Translational regulation and recruitment of stored mRNA for histones is also an important element of differentiation in early sea urchin embryos. At the fourth cleavage in embryogenesis, eight equal sized cells divide unequally to form eight mesomeres and four each macromeres and micromeres; the latter pair differ in cell volume by five-fold. We have analyzed the distribution of stored histone mRNA in these three cell types. A method was devised to allow the rapid separation of the cells from one another in high purity from large cultures. Northern blot analysis shows that the *concentration* of histone mRNA in each cell type is nearly identical, leading to the *amount* of histone message in the small cells being only 20% that in the macromeres. This is a clear example of a molecular mechanism of differentiation -the proteins synthesized in the two cells will differ during later growth even if the rate of transcription of new RNA is the same for all cells.

A model system for study of development of hormonal responsiveness is provided by virally transformed MDCK cells; response to glucagon and glucagon binding are diminished compared to the untransformed parental line. Previously we reported that the transformed cells were deficient in production of prostaglandin E_2. It now appears that the transformed cells are deficient in phospholipase activity, leading to a reduced rate of release of arachidonic acid, the major prostaglandin precursor. Similar findings are made for Kirsten MSV transformed NRK cells but not in SV40 transformed CHL cells, suggesting that this may be a consequence of RNA tumor virus transformation. Support for the possible causal role of decreased prostaglandin production in disappearance of glucagon responsiveness has been gained from studies which show that indomethacin, an inhibitor of prostaglandin synthesis, leads to a diminution in the glucagon response and the number of glucagon receptors in normal (nontransformed) MDCK cells.

A transformed rat cerebellar cell line can be induced to differentiate from neuroblasts to neuronal-like cells in culture at high density. Similar morphological changes are produced by treatment of the cells with a phosphodiesterase inhibitor or a calcium ionophore. Several biochemical correlates of neuronal differentiation are present in the cells after such treatment, including increased responses to isoproterenol and dopamine, an altered phospholipid distribution and specific tetanus toxin binding.

Lipid Transport

Much of the work of the Section on Endocrinology for the past six years has evolved from a model for lipid transport from chlyomicra in capillaries to surrounding cells. The model envisages fusion of membranes to create a continuum through which fatty acids diffuse laterally after hydrolysis of triacylglycerol by lipoprotein lipase. Reesterification can occur, as in fat cells, after transport.

The extracellular space can be imaged by exposure of tissues to tannic acid. Using this method, we can detect vesicles and channels in adipose tissue which may reflect this membrane continuity. Similar findings have been made for macrophages and 3T3-L1 fat cells in culture and intestinal absorptive cells. Since tannic acid treatment is done after fixation, the apparent vesicles do not arise from pinocytosis, but rather represent either sections of channels through cytoplasm or invaginations of plasma membrane.

For several systems that have been studied in the past year, it seems that the hydrolysis-lateral diffusion model may explain transport of fatty acids within the cell as well as between cells. It is known that increased lipid hydrolysis and mitochondrial fatty acid oxidation are consequences of stress in brown adipose tissue of young rats. The appearance of lammelar whorls and aqueous spaces are morphologic indicators of such transport. Compared to control rats, starved or cold-stressed rats showed evidence of transport from lipid lenses in endoplasmic reticulum; spaces and whorls occurred near intracellular lipid droplets, in intracellular channels, and between inner and outer mitochondrial membranes. Transport is thus formally analogous to that outlined above with the modifications that endoplasmic reticulum associated lipase initiates the process and fatty acid activation for transfer to the inner mitochondrial membrane culminates it.

Exactly analogous findings were made in proximal convuluted tubule cells of diabetic or fasting rat kidney. Kidney uses plasma free fatty acid as energy source. These findings indicate that plasma fatty acids are not directly used but rather accumulate as intracellular triacylglycerol when plasma fatty acid concentrations are high (fasting or diabetes); these are then transported to mitochondria by mechanisms like those observed in brown adipose tissue. Preliminary investigations suggest a similar mechanism of transport of lipid from micelles into intestinal absorptive cells. Finally, smooth muscle cells of a small muscular rat artery develop the characteristic pattern signalling uptake of lipid by the hydrolysis-lateral diffusion mechanism when perfused with triacylglycerol emulsions. A similar pattern of morphological observations to those documented above suggests that these cells too store, transport to mitochondria, and oxidize lipid as an energy source.

We have extended such studies to cells in culture where the extracellular environment is more easily manipulated. Macrophages synthesize and secrete lipoprotein lipase; observations that albumin increases the rate of hydrolysis of emulsified triacylglycerol added to the medium, increases (25 fold) the cellular content of triacylglycerol in such cultures, and increases the glycerol content of the medium suggest that these cells contain a functionally important monoacylglycerol lipase. Treated macrophages develop the characteristic lamellar whorls which are through to reflect accumulations of fatty acids along the continuum of cell membranes. Histochemical studies show that the whorls are not related to lysosomes in these cells. Uptake of lipid by phagocytosis occurred only when the low density lipoproteins in the medium were acetylated - normal LDL was not engulfed. More cholesteryl ester than cholesterol was taken up, although this ratio was altered and the absolute amounts changed by the presence of other lipids in the medium.

A major issue in mechanisms of lipid transport is whether ionized long chain fatty acids dissolve in the aqueous phase, on the one hand, or remain associated with a lipid phase, on the other. We have studied this by morphological observations of oleic acid/water interfaces at various pH values. At high pH (11-12) long filaments form from an oleic acid droplet and soon break off into the aqueous phase. The spreading pressure of oleic acid drops at these pH's, indicating loss of the acid from the surface. Quantitative measurement of loss of oleic acid from a droplet were carried out in a perfusion chamber. Only a 7-fold increase occurred from pH 1 to 7.4, another 12-fold by pH 9.6, and 2000-5000-fold at pH 11 to 12. Filaments begin to develop at pH 7.4 and increase in amount at pH 9.6. At the highly alkaline pH's, filaments seem to melt away from the droplet; the appearance of small short strands leaving the droplet suggested formation of lamellar whorls. The data thus suggest that most fatty acids remain at the lipid-water interface when ionization occurs and that few if any are dissolved in the aqueous phase.

Critical to future efforts in understanding this mechanism of lipid transfer is the enzymology and regulation of the lipases. We have studied two of these. Pharyngeal lipase has been purified to specific activities of 300-700 lipolytic units/mg protein by several methods; a unique approach using hydrophobic chromatography and elution with bile salt micelles is of particular interest. The molecular weight of the enzyme appears to about 50,000.

Hormonal regulation of lipoprotein lipase (LPL) has been studied in 3T3-L1 fat cells in culture using both activity measurements and measurement of immunoreactive protein (IRP). Both LPL and IRP increase when cells become confluent or upon insulin treatment. The activity is unstable in either case; IRP concentrations remain stable, however. New synthesis of LPL is stimulated by insulin; both membrane (active) and inactive (presumably preenzyme) forms are synthesized under hormonal stimulation. Insulin appears to accelerate transport of LPL from an inactive, newly synthesized form to the plasma membrane bound, active form. Several levels of regulation of LPL activity by hormone thus exist.

Features of lipid metabolism in several human disease states have been detailed during the past year. A patient with a genetic apolipoprotein A-I deficiency has an absence of HDL particles and abnormal distributions of lipoproteins in the VLDL and LDL fractions. Morphologic observations of these fractions show an abnormal particle appearance with strands of filamentous material in VLDL and LDL fractions and absence of the characteristic 70A particles of the HDL fraction. It had been suggested by others that cystic fibrosis might be characterized by a diminished response of the adenylate cyclase system to beta-adrenergic effectors. Adipocytes of patients with cystic fibrosis respond to stimulation with isoproterenol, theophylline and dibutryl cyclic AMP with increased lipolysis and glycerol release, negating the previous suggestion. Pseudohypoparathyroidism patients appear to have a defect in receptor-adenylate cyclase activity in kidney and lack a functional guanine regulatory unit in erythrocytes. Interestingly, the defect in the G-unit is tissue specific, since (1) adipocytes from patients with this disease respond to isoproterenol, theophylline or dibutryl cyclic AMP just as controls and (2) plasma glycerol increases after glucagon infusion.

Hormonal Mechanisms

Previously we have documented the duality of regulation possible for adenylate cyclase; distinct processes lead to stimulation or inhibition of the enzyme and both processes are dependent upon guanine nucleotides. Further differences in these two processes have been found using fat cell membranes. GTP decreases binding of beta adrenergic hormones (which stimulate cyclase). Divalent cations are without effect on the GTP modulation of binding. In contrast, adenosine analogs (inhibitory for cyclase activity) have their specific binding increased by GTP; in this case $MgCl_2$ altered the GTP effect in a bimodal fashion as a function of concentration. These data provide further evidence suggesting the non-identity of the GTP regulatory proteins modulating stimulation and inhibition of adenylate cyclase.

The fat cell is target for a wide variety of hormones which regulate its pivotal position in animal cell energy metabolism. For the effort that has been spent on attempts to determine the mechanisms whereby hormones alter fat cell metabolism, we know remarkably little about this critical area today. In part this is due to lack of consistency in many experiments using fat cells; we felt that a possible critical element in the irreproducibility of experiments was variability in the preparations of cells. We and others have shown that adenosine acts at nanomolar concentrations on cell surface receptors linked to adenylate cyclase. Since fat cell preparations involve proteolytic disruption of tissue and extensive washing, we reasoned that variable release and removal of adenosine might cause a portion of the variability.

It was found that acute removal of adenosine leads to a 10-fold activation of cyclic AMP-dependent protein kinase (A-kinase). Cells isolated by standard procedures exhibit highly variable A-kinase activities at various stages of the isolation procedure. Inclusion of 200 nm adenosine during washing steps allows preparation of fat cells with consistently low A-kinase activities. Adenosine can be acutely removed by enzymatic treatments later.

Using these cells, we find that the resting (inactive in lipolysis) fat cell A-kinase activity is less than 5% of the total enzymatic activity (vs. 30-40% reported previously). Lipolytic hormones increase cyclic AMP transiently and this is reflected in the activity of A-kinase. However, A-kinase activity returns to pre-stimulation levels while lipolysis continues unabated; at this point, adenosine can still inhibit lipolysis but has no effect on the residual activity of A-kinase. It seems that either adenosine has two mechanisms of inhibition of lipolysis or that the initial increase in cyclic AMP and activation of A-kinase by lipolytic hormones, previously shown to be inhibited by adenosine, is not directly related to lipolysis. Adenosine mimics many of the effects of insulin in adipocytes. The findings of these studies may thus be relevant to the mechanism whereby insulin affects energy metabolism through the fat cell.

Enzymology

Two enzymes, dihydrofolate reductase (chicken liver) and an extracellular ribonuclease (*Bacillus amyloliquefaciens*) have been studied extensively in this laboratory for over a decade. Rather remarkably, the crystal structures of both this have been determined (in collaboration with others) in the same, this, year.

The structure of dihydrofolate reductase is available at 2.9 A resolution as a complex with NADPH and an inhibitor. This is the first eukaryotic enzyme of its type whose structure has been established. Overall folding of the enzyme is similar to that seen for bacterial reductases. Many of the additional residues present in the avian enzyme occur in loops far removed from the active site region; an insertion near the amino terminal region contains the unique sulfhydryl group of the chicken protein. NADPH is held in a shallow groove across one face of the enzyme in an extended configuration. Other folate reductases are being isolated by the affinity chromatographic procedures developed in this laboratory for similar investigations; of particular interest are enzymes from human liver and a methotrexate resistant breast tumor cell line.

The ribonuclease structure is available at 2.5 A resolution. The structure evinces the remarkable stability of this small protein, with a five-stranded antiparallel β-sheet core packed by two regions of α-helix. The active site consists of a slight depression on the protein surface containing three of the five evolutionarily conserved amino acid residues. The structure of the enzyme is quite different from pancreatic ribonuclease - both in secondary structure and the organization of the active site region. We have established genomic libraries of *B. amyloliquefaciens* with the goal of cloning the genes for both this ribonuclease and its intracellular inhibitor. These should facilitate production of the proteins and allow genetic manipulation to produce mutant proteins with which to further examine the features which lead to the stability of the ribonuclease, its mechanism of action, and the bases for the specificity of enzyme-inhibitor interaction.

Hepatic and Intestinal Function

Past investigations in this laboratory have established the virtues of metabolic studies using surgically isolated, perfused organs, primarily liver and small intestine. Recently, we have focussed on the biosynthesis of apolipoproteins in the rat liver. Both the two variant forms of apolipoprotein B and the multiple variants of apolipoprotein A-1 have been studied. In collaboration with scientists of the NHLBI, a number of new methodological approaches to the analysis of the lipoproteins have been developed. Using these, we have shown that the relative amounts of the two apolipoproteins synthesized in the liver is influenced by the diet fed to animals. Both proteins are incorporated into VLDL and significant amounts of the smaller variants appear in HDL. Of interest, the perfused rat liver synthesized all the variant forms of the apolipoproteins, but not in the proportions which are observed in serum; specifically, very little of the most abundant serum form was produced. This suggests that the major apolipoprotein A-1 isoproteins may be differentially metabolized by extrahepatic tissues.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 15001-08 LCDB

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)
Structure-Function Relationship of Glucagon

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	David E. Wright	Senior Staff Fellow	LCDB	NIADDK
	Martin Rodbell	Section Head	LCDB	NIADDK

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Cellular and Developmental Biology

SECTION
Membrane Regulation Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	0

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is terminated.

PHS-6040

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AM 15004-07 LCDB

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Regulation of Hormone Responsiveness During Cellular Development

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	Suzanne K. Beckner	Senior Staff Fellow	LCDB	NIADDK
	Michael C. Lin	Research Chemist	LCDB	NIADDK
Other:	Robert Littlejohn	Chemist	LCDB	NIADDK

COOPERATING UNITS (if any)
High School Student Volunteers

LAB/BRANCH
Laboratory of Cellular and Developmental Biology

SECTION
Membrane Regulation Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.3	0.7

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The de novo synthesis of glucagon receptors can be induced in transformed MDCK cells by butyrate (a known differentiation inducer) and prostaglandin E_1 (which elevates cAMP in these cells) and RO20-1724 (an inhibitor of phosphodiesterase). However, the concentration dependence of induction for both prostaglandin E_1 and RO20-1724 do not parallel their ability to elevate cAMP levels. In fact, higher concentrations of cAMP actually inhibit the induction process, although there is no effect of high cAMP concentrations on glucagon responsiveness of the parental MDCK cell line. Additional studies, utilizing glucagon fragments suggest that the newly synthesized glucagon receptors of transformed cells are similar but not identical to those of the parental line. The data suggests that high levels of cAMP inhibit the synthesis of glucagon receptors and that important differences in the newly synthesized receptor system may provide insight into the consequences of viral transformation.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 15005-07 LCDB

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Actions of Adenosine and Adenylate Cyclase

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	Constantine Londos	Research Chemist	LCDB	NIADDK
Other:	Rupert Honnor	Visiting Fellow	LCDB	NIADDK
	Gurpreet Dhillon	Staff Fellow	LCDB	NIADDK

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Cellular and Developmental Biology

SECTION
Membrane Regulation Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	2.5	0.5

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Adipocyte preparation and extraction procedures have been developed to allow demonstration of important mechanisms of inhibition of lipolysis by adenosine. An improved assay for adipocyte cyclic AMP dependent protein kinase (A-kinase) has been developed, which allows for accurate measurement of the cellular state of activation of A-kinase.

Acute removal of adenosine (using adenosine deaminase) from adipocyte preparations is found to lead to rapid, transient activation of A-kinase. In the quiescent, resting state (inhibited by nanomolar concentrations of adenosine) adipocytes show a very low fractional activation of A-kinase, much lower than reported heretofore. After the activation of adipocyte A-kinase, by removal of adenosine, has relaxed to resting levels, lipolysis still continues at linear rates and may be inhibited by the addition of the adenosine-analog N^6-phenylisopropyladenosine in an apparently cyclic AMP- and A-kinase-independent manner.

PHS-6040

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 15010-04 LCDB

PERIOD COVERED

October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

GTP-mediated Stimulation and Inhibition of Adenylate Cyclase

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	Dermot M.F. Cooper	Visiting Associate	LCDB	NIADDK
Other:	Donald L. Gill	Visiting Fellow	LCDB	NIADDK
	Martin Rodbell	Section Chief	LCDB	NIADDK

COOPERATING UNITS (if any)

Rosemary Jagus, Visiting Associate, LMH, NLHBI
Robert L. Somers, Biologist, LVR, NEI

LAB/BRANCH

Laboratory of Cellular and Developmental Biology

SECTION

Section on Membrane Regulation

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The regulation of binding to stimulatory versus inhibitory receptors by GTP and divalent cations has been compared in fat cell membranes. The contrast in the regulation of binding to the two classes of receptor lends further support to the notion of discrete GTP regulatory proteins in association with receptors which mediate stimulation or inhibition of adenylate cyclase.

Complementation/fusion studies have been carried out between systems replete in dual regulatory components and those which lack either inhibitory receptors or inhibitory GTP regulatory proteins. These studies offer firm evidence of the existence of discrete stimulatory and inhibitory GTP regulatory proteins and provide assay systems for these components, which will permit their purification and characterisation.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 15100-12 LCDB

PERIOD COVERED

October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Protein-Nucleic Acid Interactions: Chromatin Structure and Function

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	Robert T. Simpson	Medical Director (R)(T)	LCDB	NIADDK
Others:	Lawrence W. Bergman	Senior Staff Fellow	LCDB	NIADDK
	Tauseef Butt	Visiting Fellow	LCDB	NIADDK
	Fritz Thoma	Visiting Fellow	LCDB	NIADDK
	Malabi Venkatesan	Staff Fellow	LCDB	NIADDK
	Tzuchen Wu	Chemist	LCDB	NIADDK

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Cellular and Developmental Biology

SECTION

Section on Developmental Biochemistry

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.0	5.0	0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We have studied chromatin structure of regulated genes in several systems. Cloning of chromatin is possible in yeast - as a first example we have defined the structure of a minichromosome containing the TRP1/ARS1 fragment of yeast DNA. Nine precisely phased nucleosomes are associated with the 1453 base pair length of DNA. In the yeast acid phosphatase gene, phased nucleosomes are present on the transcribed segment when the gene is inactive but phasing does not exist when the gene is actively transcribed. Regions outside the transcribed segment have phased nucleosomes in either case. For both genes, DNAase I hypersensitive sites are located near the 5' end of the gene when active transcription is occurring and absent when the gene is repressed. Qualitatively similar conclusions are made from studies of the early sea urchin histone genes during development. The basis for nucleosome phasing has been determined in one case, the association of histones with a cloned sea urchin DNA segment containing a 5S rRNA gene. Precise positioning of histones on DNA is determined by these two species alone. Detailed examination of this nucleosome reveals that the two DNA strands in the core particle are not identical in their interactions with histones.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 15102-22 LCDB

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)
Study of a Ribonuclease and its Inhibitor from Bacillus amyloliquefaciens

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	Robert W. Hartley	Research Physicist	LCDB NIADDK

COOPERATING UNITS (if any)
C. Chothia, MRC Laboratory of Molecular Biology, Cambridge; G.G. Dodson, Dept. of Chem. University of York; Y. Mauguen, Centre Pharmaceutrique, Univ. Paris-Sud

LAB/BRANCH
Laboratory of Cellular and Developmental Biology

SECTION
Developmental Biochemistry Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.4	1.0	0.4

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Two proteins, barnase, the extracellular ribonuclease of Bacillus amyloliquefaciens, and barstar, its intracellular inhibitor, are being developed as a model system for the study of protein folding and protein-protein interactions. Barnase is one of an homologous group of ribonucleases occurring in both prokaryotes and eukaryotes. Amino acid sequence determination and X-ray structure characterization are being carried out along with thermodynamic studies of folding reactions. A peptide complementation system for barnase has been developed which will be used to investigate the roles of specific residues in both folding and enzymatic activity.

Recombinant DNA techniques are being applied to the project with three major aims: 1) to facilitate production, 2) to examine the structural and control sequences of the genes and 3) to tailor specifically designed modifications in the sequences to test theories of protein folding.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 15105-06 LCDB

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

A Developmental Transition in Mosses as a Model of Eukaryotic Genome Control

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	Robert W. Hartley	Research Physicist	LCDB NIADDK

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Cellular and Developmental Biology

SECTION
Developmental Biochemistry Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0.0	PROFESSIONAL:	OTHER:

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is inactive.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 15200-22 LCDB

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Studies on Folic Acid (Dihydrofolate Reductase)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	Bernard T. Kaufman	Research Chemist	LCDB NIADDK

COOPERATING UNITS (if any)
Dr. Neil Clendeninn, Cancer Institute, DCT; and Dr. David Matthews, University of California, San Diego

LAB/BRANCH
Laboratory of Cellular and Developmental Biology

SECTION
Nutritional Biochemistry Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

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☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Dihydrofolate reductase is the site of action of several important drugs including trimethoprin, pyrimethamine and the widely used cancer chemotherapeutic agent methotrexate. We attempt to understand the mechanism of action as well as inhibition of this enzyme and also to elucidate its molecular genetics. Despite small amounts of the enzyme in normal animal tissues and the resulting problems associated with purification and isolation of a homogeneous crystalline enzyme under these conditions, we can now report the 2.9 Å structure of the chicken liver dihydrofolate reductase in a ternary complex with NADPH and a phenyltriazine inhibitor - the first structural determination of dihydrofolate reductase from a eukaryotic species. The same purification procedure, affinity chromatography and isoelectric focusing, is currently being used in an attempt to isolate the reductase from certain human tissues, however these studies are complicated by even smaller levels of enzyme activity than previously observed in avian and bovine liver.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 15201-03 LCDB

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Ligand Interactions with Proteins: Model Studies with Cibacron Blue: Physico-Chemical Studies

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	S. Subramanian	Visiting Scientist	LCDB	NIADDK
Other:	Bernard T. Kaufman	Research Chemist	LCDB	NIADDK

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Cellular and Developmental Biology

SECTION
Nutritional Biochemistry Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Dihydrofolate reductase binds the dye Cibacron Blue F3GA. In order to understand the nature of this interaction since it might yield information concerning the interaction of this enzyme with its substrates and inhibitors, i.e., methotrexate, model interactions of Cibacron Blue with organic solvents, salts, oligopeptides, and polypeptides were studied by visible difference spectroscopy. The difference spectrum of the dye under ionic conditions has a characteristic positive peak at 690 nm and negative double minima at 630 and 585 nm. Similar "salt-like" spectrum were also obtained with a series of polycations and protamine. In contrast, the difference spectra obtained under nonpolar conditions displays a positive peak and shoulder at 655 and 610 nm with a small negative contribution below 550 nm. The spectral characteristics described here for the interaction of the polyaromatic polysulfonate dye with various charged, polar and nonpolar groups may provide a basis for characterizing the binding environments in proteins in general as well as dihydrofolate reductose.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 15202-03 LCDB

PERIOD COVERED

October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Thermodynamics of Dehydrogenase-Ligand Interactions

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	S. Subramanian	Visiting Scientist	LCDB NIADDK

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Cellular and Developmental Biology

SECTION

Nutritional Biochemistry Section

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is terminated

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 15300-17

PERIOD COVERED
October 1, 1981 through December 31, 1981

TITLE OF PROJECT (80 characters or less)

Biochemical Studies Related to Nutrition

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: John G. Bieri, LCDB, NIADDK

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Cellular and Developmental Biology

SECTION
Nutritional Biochemistry Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project has been terminated.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 15302-12 LCDB

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Biochemical Studies of Hepatic and Intestinal Function

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Dr. H.G. Windmueller	Research Chemist	LCDB NIADDK
OTHER:	Dr. Mary B. Sliwkowski	Staff Fellow	LCDB NIADDK
	Mr. A.E. Spaeth	Chemist	LCDB NIADDK

COOPERATING UNITS (if any)
Drs. Giancarlo Ghiselli and H. Bryan Brewer, Jr., Molecular Diseases Branch, NHLBI

LAB/BRANCH
Laboratory of Cellular and Developmental Biology

SECTION
Nutritional Biochemistry Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 2.3	PROFESSIONAL: 1.3	OTHER: 1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Biosynthesis of the two variant forms of plasma apolipoprotein B and the multiple isoforms of plasma apolipoprotein A-I was studied in perfused rat livers. The relative amounts of the two apolipoprotein B variants produced was influenced by the diet, with implications for the metabolic fate of the associated lipoproteins. Both newly-synthesized variants were incorporated into all sizes of very low density lipoproteins produced, with little segregation into different sized particles. Significant amounts of the smaller variants sometimes appeared in high-density lipoproteins. Hepatic release of the smaller variant was relatively delayed, suggesting a difference in the assembly or release of lipoprotein particles containing the two proteins. Apolipoprotein A-I, the major protein of serum high-density lipoproteins, was found to exhibit polymorphism, circulating in serum as two major isoproteins and several minor species. Rat liver synthesized all of the isoproteins, but not in the proportions observed in serum. Very little of the most abundant serum isoform was produced. These findings suggest that the two major apolipoprotein A-I isoforms may be interconverted by extra-hepatic tissues or may differ in their site of biosynthesis or in their catabolic rate.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-AM-15400-08 LCDB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Hormones, Lipoprotein Lipase and Lipid Metabolism

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Robert O. Scow	Chief, Section on Endocrinology	LCDB NIADDK
OTHERS:	Sidney S. Chernick	Scientist Director	LCDB NIADDK
	Thomas Olivecrona	Visiting Scientist	LCDB NIADDK
	Thomas Briggs	Guest Worker	LCDB NIADDK

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Cellular and Developmental Biology

SECTION
Section on Endocrinology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 3.0	PROFESSIONAL: 1.2	OTHER: 1.8

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We are using 3T3-L1 fat cells for studying hormonal regulation of lipoprotein lipase (LPL) in cells. We recently developed a radioimmunoassay for the enzyme, which allows comparison of the amount of lipolytic activity with the amount of immunoreactive protein (IRP) present in cells and in the medium under various conditions. Our findings indicate IRP probably exists in two forms in the cells: one that is releasable by heparin and had lipolytic activity, and another that is not releasable by heparin, has little or no lipolytic activity, is probably membrane associated, and is assayed as IRP only after being solubilized by SDS. We earlier reported that LPL activity in the medium is increased when cells are treated with insulin. Our recent findings show this increase in LPL activity is paralleled by a comparable increase in IRP. Studies with radioactive methionine showed that insulin stimulates synthesis of lipoprotein lipase. Cyclohexamide prevented increases in LPL activity and IRP, but did not affect the amount of LPL activity released from cells by heparin. Incubation with insulin for 1 hr increased 5-fold the amount of LPL activity released from cells by heparin, suggesting that insulin is also involved in transport of the enzyme within fat cells.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-AM-15401-10 LCDB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Transport of Lipids, Hormones and Enzymes in Tissues, Cells and Membranes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Robert O. Scow	Chief, Section on Endocrinology	LCDB NIADDK
OTHERS:	E. Joan Blanchette-Mackie	Research Biologist	LCDB NIADDK
	Mary G. Wetzel	Staff Fellow	LCDB NIADDK
	Alli Reinila	Visiting Fellow	LCDB NIADDK

COOPERATING UNITS (if any)
Ernst Schaefer, MD, NHLBI; Bryan Brewer, Jr., MD, NHLBI

LAB/BRANCH
Laboratory of Cellular and Developmental Biology

SECTION
Section on Endocrinology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.0	2.9	2.1

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Our group has developed a model for lipid transport between blood and the interior of cells in tissues by lateral movement in an interfacial continuum of the outer leaflets of various cell membranes. Our studies in white adipose tissue and heart, reported last year, gave morphological evidence for an interfacial continuum extending from the luminal surface of capillaries to lipid droplets and mitochondria in parenchymal cells. Recent studies in brown adipose tissue and kidney demonstrate that the membrane of endoplasmic reticulum is continuous with the outer mitochondrial membrane. Thus, fatty acids formed by action of tissue (hormone-sensitive) lipase on intracellular triacylglycerol can enter the continuum at sites of lipolysis in endoplasmic reticulum and move in the continuum to the outer mitochondrial membrane where they can be activated for transfer to the inner matrix for oxidation. Phase microscopic and radiochemical analyses showed that ionization of long chain fatty acids at pH 7.4 to 9.6 causes rapid enlargement of the oil-water interface and very little loss of fatty acids to aqueous medium, whereas at pH 11 to 12 the rate of growth of the interface is much faster and some of the fatty acids are lost, many as strands, to the aqueous perifusate.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-AM-15402-07 LCDB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Pharyngeal Lipase and Digestion of Dietary Fat

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Robert O. Scow	Chief, Section on Endocrinology	LCDB NIADDK
OTHER:	Ruth B. Field	Guest Worker	LCDB NIADDK

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Cellular and Developmental Biology

SECTION
Section on Endocrinology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 1.2	PROFESSIONAL: 1.2	OTHER: 0

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Pharyngeal lipase is secreted in the pharynx of man and rat, and it acts in the stomach at pH 5.4 to hydrolyze dietary triacylglycerol to diacylglycerol, monoacylglycerol and fatty acids. These amphipathic products facilitate emulsification of lipid in the stomach before it passes to the small intestines for further digestion and absorption. We have continued our effort to purify this enzyme from rat tongue and have succeeded in obtaining a nearly pure preparation. A protein preparation with lipolytic activity has been obtained from a homogenate of rat lingual serous glands by precipitating from a 100,000 x g supernatant with 60% saturated ammonium sulfate solution, resuspending in aqueous buffer (pH 5.4) and precipitating with cold acetone; this fraction contained 110 units of lipolytic activity per mg. Further purification yielding a specific activity of 700 units per mg was obtained by passing the above protein preparation through the cationic exchange resin SE sephadex G-25. Gel filtration indicates the molecular weight of the enzyme is 48,000 daltons. Protein eluted from a hydrophobic column of ethyl agarose showed one major band, with a molecular weight of 50,000 daltons, on SDS-polyacylamide gel electrophoresis.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 15403-04 LCDB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Diabetes, Insulin and Blood Vessels

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Robert O. Scow Chief, Section on Endocrinology, LCDB NIADDK

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Cellular and Developmental Biology

SECTION
Section on Endocrinology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

INACTIVE

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-AM-15404-01 LCDB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Sensitivity of Human Adipocytes to Hormones in Genetic Diseases

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Sidney S. Chernick	Scientist Director	LCDB NIADDK

COOPERATING UNITS (if any)
M.H. Levine, MD, NIADDK; G.D. Aurbach, MD, NIADDK; M.S. Chernick, PM, NIADDK; P.A. di Sant'Agnese, PM, NIADDK

LAB/BRANCH
Laboratory of Cellular and Developmental Biology

SECTION
Section on Endocrinology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0.5	PROFESSIONAL: 0.5	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Adipocytes isolated from adipose tissue obtained by needle biopsy have been used to explore the genetic defects in Albright's Hereditary Osteodystrophy (pseudohypoparathyroidism, PsHP), and Cystic Fibrosis. Despite a defect in the guanine nucleotide regulatory protein of the receptor-adenylate cyclase complex in erythrocytes of PsHP patients, no lack of sensitivity to isoproterenal was found in adipocytes of the same patients. This is consistent with our observation that these patients have a normal rise in plasma glycerol concentration after glucagon administration. The unique obesity of Albright's Hereditary Osteodystrophy is not explainable as a genetic defect in lipid mobilization due to failure of the receptor-adenylate cyclase complex. Cystic Fibrosis (CF) is a genetic disease without an accepted biochemical basis. It had been suggested that impaired responsiveness to sympathomimetic amines implicate the beta-adrenergic receptor--adenylate cyclase complex as the site of a systemic genetic defect. However, adipocytes of CF patients responded normally to isoproterenol indicating that these cells are not covered by this hypothesis. The responsiveness of adipocytes to hormones, the simplicity and low risk of needle biopsy make this an ideal method for clinical studies.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 15500-22 LCDB

PERIOD COVERED

October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Large-scale Processing of Biological Materials

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	Joseph Shiloach	Head, Pilot Plant	LCDB NIADDK

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Cellular and Developmental Biology

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The Pilot Plant is involved in large scale growth of micro-organisms and mammalian cells, and in large scale isolation and purification of biologically active components from these sources and from other sources such as mammalian or plant tissues. We assist NIH investigators in the scaling up of processes by checking different approaches and different methods suitable for large scale processing.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 15501-02 LCDB

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)
Role of Prostaglandin Synthesis in Differentiation and Transformation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	Michael C. Lin	Research Chemist	LCDB	NIADDK
	Suzanne K. Beckner	Senior Staff Fellow	LCDB	NIADDK
Other:	Sue-May Wang	Visiting Fellow	LCDB	NIADDK
	Robert Littlejohn	Chemist	LCDB	NIADDK

COOPERATING UNITS (if any)
Vincent Manganiello, Section Chief, LCM, NHLBI

LAB/BRANCH
Laboratory of Cellular and Developmental Biology

SECTION
Membrane Regulation Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 2.0	PROFESSIONAL: 2.0	OTHER: 0.3

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Our observation that glucagon sensitivity is lost and prostaglandin (PG) production reduced in MDCK cells upon viral transformation, but could be restored by culturing cells in the presence of PG, led to our hypothesis that PG serve as locally produced developmental hormones. Reduced production results from decreased activities of phospholipase and synthetase in transformed cells. Additionally, glucagon responsiveness is reduced in normal MDCK cells when PG production is inhibited. We are currently trying to determine if the development of glucagon receptors in MDCK cells is directly related to PG production. Reduced PG production was also observed in NRK cells transformed with Kirstem sarcoma virus, but not in CHL cells transformed with SV40 virus. Whether decreased PG production is a unique characteristic of transformation by RNA tumor viruses is currently under investigation.

Our collaborations with V. Manganiello revealed that alterations is transformed MDCK cell phosphodiesterase activities could be restored by differentiation inducers suggesting that these cells serve as a useful model for studying the development of various cellular functions.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 15502-01 LCDB

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Regulation of Neuronal Differentiation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	Suzanne K. Beckner	Senior Staff Fellow	LCDB	NIADDK
Others:	Hayden G. Coon	Research Biologist	LCB	NCI
	Peter H. Fishman	Research Chemist	DMNB	NINCDS
	Norman Salem	Senior Staff Fellow	DMNB	NINCDS

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Cellular and Developmental Biology

SECTION
Membrane Regulation Section

INSTITUTE AND LOCATION
NIAMDD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0.4	PROFESSIONAL: 0.3	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The differentiation of neuroblasts into neurons involves morphological as well as biochemical changes, a process which is poorly understood. A cloned cell line of undifferentiated neuroblasts (CBSV) has been found to differentiate spontaneously under defined conditions, but can also be induced to undergo morphological change by calcium ionophore and isobutylmethyl xanthine (an inhibitor of phosphodiesterase)under non-differentiating conditions. This chemical induction of differentiation is accompanied by increases in neurotransmitter-responsive adenylate cyclase activity and membrane phospholipids more characteristic of adult neuronal tissue. The ganglioside pattern of both differentiated and undifferentiated CBSV cells is similar, neither having more complex gangliosides characteristic of normal neurons. However, chemical induction of differentiation results in the appearance of specific tetanus toxin binding, usually taken as a specific neuronal marker. These data suggest that CBSV cells represent a good model system for examining factors which regulate normal neuronal differentiation during development.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 15503-01 LCDB

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Regulation of Developmental Gene Expression

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	Alan R. Kimmel	Special Expert	LCDB	NIADDK

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Cellular and Developmental Biology

SECTION
Developmental Biochemistry Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0.9	PROFESSIONAL: 0.9	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

M4 is a short, transcribed repeat sequence interspersed with singly-copy DNA in the Dictyostelium genome. I have isolated a set of cloned mRNAs (cDNAs) which contain M4 sequences covalently linked to different single-copy sequences. The M4 repeat lies at the 5'-end of these cDNAs. During Dictyostelium development, the expression of the M4 sequences increase > 5-fold relative to vegetative growth. Additionally, using single-copy probes to specific M4 mRNAs, I have shown that these individual mRNAs exhibit a relative increase in their developmental expression which is similar to that of the total M4 mRNA population. There may be a co-ordinate increase in expression of the M4 gene set during development.

I have additionally, identified an independent transcription-unit whose expression rapidly decreases at the onset of development. Interestingly, this gene lies within 1500 base pairs of a gene with a completely opposite pattern of developmental expression.

ANNUAL REPORT OF THE

LABORATORY OF BIOCHEMISTRY AND METABOLISM

A. Polysaccharide Participation in Morphogenesis.

1. A study on the distribution of chitin in the cell wall of several temperature-sensitive cell cycle mutants was continued. The results show that cell cycle arrest in most of these mutants leads to a random deposition of chitin in the cell wall rather than the sharp localization in the septum, as observed in wild-type cells or in the mutants at a permissive temperature. It is concluded that the mechanism that normally insures localized chitin synthesis is highly dependent on cell organization and can be easily disrupted. Completion of this work awaits some electron microscopy of control cells.

The previous assumption that proteinase B was the physiological agent for activation of chitin synthetase zymogen was proved incorrect by the isolation of mutants that lack proteinase B, yet show normal synthesis of chitin and septum formation in vivo. Although extracts of such mutants were found to catalyze a slow activation of the zymogen, apparently by a proteolytic mechanism, the results were not reproducible and ultimately this approach had to be abandoned. We came back then to the older observation that treatment of protease B-deficient cells with digitonin leads not only to permeabilization of the cells but also to considerable and rapid activation of chitin synthetase. The conditions for activation were studied in detail and the following conclusions were reached: a) the activation of of chitin synthetase is a temperature and time-dependent process, distinct from mere permeabilization of the cells; b) the activation is inhibited by certain protease inhibitors, such as antipain and leupeptin, but not by others, such as chymostatin, pepstatin and phenylmethylsulfonyl fluoride, c) the chelating agents, EDTA and EGTA, inhibit the activation; Ca^{2+}, but not Mg2+, reverses this effect, d) the activated synthetase differs from that obtained by treatment of the zymogen with trypsin in some of its properties, such as dependence on pH and on free N-acetylglucosamine.

These results implicate a previously undetected protease in the process of activation but do not establish whether the modification of the synthetase is itself proteolytic in nature. A paper embodying these results is in preparation. Efforts are now being made to obtain activation of the synthetase in cell-free extracts, to identify the specific steps involved in this process.

2. In the course of studies on chitin formation, it became necessary on many occasions to use chitinase in order to analyze chitin in cell walls or to hydrolyze the polysaccharide formed in an enzymatic reaction. Commercial preparations of chitinase are very impure and of low activity and extracts of Streptomyces, which were used previously, are difficult to purify. A search of the literature revealed that certain strains of Serrata marcescens excrete a large amount of chitinase in the growth medium. With the use of such a strain, a simple purification of chitinase was devised and the purified enzyme was characterized. The kinetic properties of the chitinase turned out to be appropriate for its use as an analytical tool. A paper summarizing these results has been submitted for publication.

B. Hormonal-Dependent Development of Mammary Gland.

1. Previous studies which strongly suggested that insulin plays a vital role in selective gene expression in mouse mammary epithelial cells have been extended. Further studies on rat mammary cells *in vitro* indicate that:

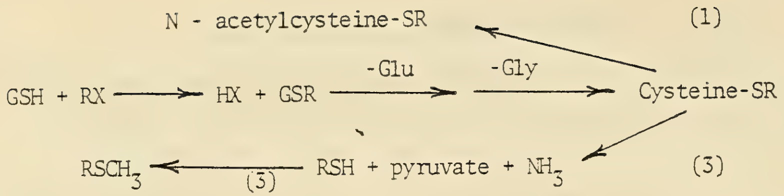
- a) in the absence of insulin neither alpha-lactalbumin nor casein gene expression occurs
- b) insulin is effective at physiological concentration
- c) EGF, MSA, FGF, NGF and PDGF are ineffective
- d) the role of insulin is independent of any role in cell maintenance

In the context of cell maintenance, it was observed that no alpha-lactalbumin gene expression occurs during 3 days of culture in the presence of MSA, glucocorticoid and prolactin. However, when insulin is added at the end of the third day, a prompt response occurs. Other reports suggest that insulin may also play a vital role in the development of certain other cell types. We propose that, in addition to being a metabolic and growth factor, insulin may also serve as a "developmental" hormone which is essential for selective gene expression in certain cells at particular stages of ontogeny.

2. It was reported last year that insulin and glucocorticoid can selectively induce alpha-lactalbumin in mammary explants from pregnant rats in the absence of prolactin. It was observed more recently that a factor present in crystallized BSA greatly enhances this induction. Under certain conditions, prolactin can antagonize this effect of BSA-factor.

C. Enzymatic Basis of Detoxication.

1. The detoxication pathway of mercapturate formation leads from the original thioether formed between an electrophile and glutathione, through the respective removal of q-glutamyl and glycine groups, leaving a thioether of cysteine which is acetylated to form an *N*-acetylcysteine thioether, i.e., a mercapturic acid. The first three steps along this path are well established with each catalyst available in homogeneous form. The subsequent acetylation step is carried out by a membrane protein, an *N*-acetyltransferase (Eq. 1), and had not been characterized. To complicate matters, there is a shunt operative by which the non-acetylated cysteine derivative is diverted to yield a thiol in a reaction catalyzed by cysteine conjugate β-lyase (Eq. 2). Thiols are highly toxic but a means for removal is available (Eq. 3); thiol *S*-methyltransferase can accomplish that task.



Each of the three mentioned enzymes has now been purified from rat tissues, the last two to homogeneity by the criterion of disc gel electrophoresis. The cysteine S-conjugate *N*-acetyltransferase has been solubilized, partially purified, and characterization to justify the degree of specificity in the proposed enzyme name. Cysteine conjugate β-lyase has been purified and shown to carry out a cystathionase type of reaction with both aliphatic and aromatic *S*-cysteine derivatives to form a thiol, pyruvate and ammonia. *Parenthetically*, the lyase can use as substrate dichlorovinylcysteine, the toxic metabolite of 1, 1, 2-trichlomethylene. Thiol *S*-methyltransferase, also solubilized from membranes and in a homogeneous *state*, is the catalyst for the transfer of a methyl group from *S*-adenosylmethionine to aliphatic and aromatic mercaptans.

Each of the purified enzymes is characterized by relatively low specificity, high K_m, and low catalytic efficiency, as might be expected of enzymes "designed" for removal of the large number of foreign compounds that are encountered.

D. Differentiation of Lymphoid Cells.

1. The addition of T cells to accessory cells (peritoneal exudate or B cell enriched populations) previously incubated with antigen resulted in activation of T cells. On the other hand, addition of antigen alone to T cells at a concentration equivalent to that associated with accessory cells was essentially without effect. When chloroquine (known to elevate the pH of primary endocytic vesicles as well as lysosomal vesicles) was added to accessory cells alone with antigen, there was a marked inhibition in the ability of accessory cells to present antigen to T cells. Addition of chloroquine to accessory cells some hours after incubation with antigen resulted in accessory cell populations that were uninhibited as regards their capability to present antigen. These findings suggest that accessory cells may have a role in processing soluble antigen prior to its presentation to T cells.

2. A comparison of splenocytes and peripheral blood lymphocytes with regard to anti-immunoglobulin and antigen activation revealed distinctions in their physical and functional behavior. Thus, splenocytes but not peripheral blood lymphocytes were substantially inhibited in terms of activation by anti-immunoglobulin subsequent to the absorption and elution from anti-immunoglobulin coated petri plates at 4°C. There was a three to four-fold greater concentration of anti-immunoglobulin required to achieve one-half maximal activation of splenocytes than peripheral blood lymphocytes. Subsequent to a single injection of ovalbumin or dinitrophenyl-protein *i.v.*, a much greater activation by antigen *in vitro*, was detected with splenocytes than with peripheral blood lymphocytes.

E. Hepatic Endocytosis of Serum Glycoproteins.

1. Endocytosis leading to degradation of galactose-terminated glycoproteins was examined in freshly isolated hepatocytes. Uptake was monitored as a function of time at 37°C. Experimental values were determined for the rates of internalization, dissociation of the receptor-ligand complex and degradation of the labeled ligand. Compartmental analysis and computer modeling revealed that the data were compatible with dissociation of ligand from receptor preceding ligand degradation. Ligand internalization did not result in concomitant depletion in the total number of cell surface receptors. The results of this study were interpreted to indicate that (a) ligand remains associated with receptor after internalization (b) complex is dissociated prior to degradation and (c) new unoccupied receptors are promptly returned to the cell surface from an internal pool.

2. It was shown originally that the purified receptor in rat liver was composed of those subunits as revealed by SDS gel electrophoresis. To resolve the controversy as to whether the three bands were functionally related, a monoclonal antibody was prepared which recognized the solubilized receptor and bound to the exterior surface of isolated rat hepatocytes. The binding of this antibody to hepatocytes prevented subsequent binding of the ligand, ^{125}I-ASOR. Conversely, occupation of the receptor with ligand inhibited binding of the radioactive antibody. The secondary structure of the receptor was shown to be critical for recognition since denaturation of the receptor with 1% SDS abolished antibody binding. However, using less denaturing conditions, antigenic reactivity was retained. Under the latter conditions, preparative electrophoresis demonstrated that a unique determinant was present in each of the three polypeptides.

3. Current studies are in progress on the role of coated vesicles during the internalization of ligand by the perfused rat liver. Preliminary results indicate that both gold-labeled ligand and functionally active receptor are present in highly purified preparations isolated from the rat liver after perfusion.

F. Intracellular Sorting and Movement of Proteins.

1. Transferrin - Diferric and monoferric human transferrin bind to the surface of K562 cells. There are approximately 1.8-10^5 receptors/cell surface exhibiting a K_D of $\sim 10^{-9}$M. These represent about 20% of the total cell transferrin receptors. At 37°C cells internalize receptor-bound transferrin. These is a one for one down regulation of surface receptors with internalization. Receptors are replaced however to prevent loss of receptors below 30% of the initial value. The transferrin enters a light intracellular compartment distinct from lysosomes as shown by Percoll density gradient centrifugation. Fluorescented transferrin allowed us to obtain a measure of the pH of this intracellular compartment and it was found to be around 5.4. After a minimum of 4-5 minutes within the cell, transferrin leaves the cell undegraded. It has lost both irons which are found bound to cytosolic ferritin.

2. Ovalbumin - Nascent polypeptide chains of ovalbumin were synthesized in a rabbit reticulocyte lysate cell free translation system directed by purified ovalbumin mRNA. We examined the interaction of these peptides with microsomes during and after translation and established that it was the amino terminus of ovalbumin that initiated interaction with the endoplasmic reticulum. In contrast to all other secreted proteins ovalbumin sequesters its leader sequence during folding rather than its being proteolytically cleared.

3. Conformation of leader sequences - In order to relate the function of leader sequences, their 3-D structure, we developed an algorithm to apply conformational energy minimization to calculate the predicted 3-D structure of leader sequences. This algorithm was based on the ability to sequentially build up a peptide from component di- and tri-peptides by combining the minimum energy structures for these components. Because these sequences are highly hydrophobic we only used non-degenerate (i.e. different backbone) conformations which greatly reduced the computational task. To test our algorithm we calculated the structure of bee venom, melittin, which is structurally quite similar to leader sequences and whose X-ray structure has recently been solved. Our calculated structure fit quite well with the experimental data.

G. Function of Bacterial Outer Membrane Components.

1. The structural gene for Protein K has been cloned. Starting with hind III restriction fragments of DNA prepared from a pathogenic E. coli K1 strain, and a phage lambda vector, a lysogen was prepared in which the prophage carried the appropriate DNA sequence.

It was shown that protein K is a porin. It is related, chemically and antigenetically, to other porin proteins and functions to increase the rate of passive diffusion of nutrients and antibiotics across the outer membrane barrier.

It was also shown that porin is required for biosynthesis of the E. coli K1 capsule. Any one of several porin proteins (OmpF, OmpC, PhoE or K protein) allows capsule synthesis in an equivalent amount and at an equivalent rate to that seen in the wild-type strain. Biosynthesis of the K1 capsule by cells lacking detectable amounts of porin protein leads to cell death.

Protein K was found to be under the control of "ompB" genes. The "ompB" region of the chromosome contains at least three genes; ompR1, ompR2, and envZ. These genes regulate the relative and absolute amounts of the OmpF and OmpC porins in response to environmental conditions such as osmolarity. We found protein K was regulated by each of the three genes in a unique manner relative to the regulation of other porins.

H. Enzymes of Carbohydrate Metabolism in Invertebrates and Vertebrates.

1. A study of the pathways of utilization of blood glucose and its incorporation into tissue glycogen in Limulus has been completed. As much as 70% of Tritium administered on C-6 of glucose is excreted as ^3H_2O within 3 hours, showing an extensive rapid glycolytic degradation of glucose in Limulus. About 80% of the ^{14}C found in the blood 1 hour after injection of glucose-^{14}C is in the anionic fraction. Preferential loss of carbon from C-1 of glucose prior to incorporation into glycogen in paired *in vivo* experiments indicates a significant occurrence of the oxidative (pentose phosphate) pathway of glucose metabolism.

2. Glucose is much more rapidly and extensively incorporated into glycogen of muscle than into that of hepatopancreas, indicative of the relative importance of muscle in metabolic turnover in Limulus. Little gluconegenesis from lactate or pyruvate was observed. The glucose of Limulus blood, occurring normally at levels of only 3-5 mg per 100ml and thought by others to be of little metabolic importance, has been found to undergo rapid metabolic changes and utilization.

I. Thermodynamic and Kinetic Studies of Protein Structure.

1. Refolding of swine pepsinogen from its high pH or urea unfolded forms has been shown to occur through rapid formation of partially folded intermediates. These are converted to the native structure through slow reactions which involve proline isomerization. Previous studies showed these intermediates to have no potential pepsin activity and to have buried up to 80% of those aromatic side chains which are inaccessible to solvent in the native state.

2. These observations have been supplemented by other techniques which give complementary information as their structure. Fluorescence measurements during refolding indicate that only 30% of the proteins tryptophans are buried in the folding intermediates, in contrast to the above results. Circular dichroism experiments show the intermediates to have extensive secondary structure, (17% α helix and 30% β sheet, i.e., 80% of the structure found in the fully folded form). Hydrogen exchange studies show that native pepsinogen has a core of 120 amide protons which are involved in stable structures in the protein, which do not exchange with solvent over a 24 hour period. However, up to 70% of these normally stable protons are rapidly exchangeable in the intermediates.

3. These contrasting results can be reconciled by recalling that absorbance and circular dichroism studies give time averaged measurements of the protein's structure, while fluorescence and hydrogen exchange the dynamic measurements of structural fluctuations which allow the partial access to solvent. In the case of pepsinogen, the presence of "wrong" proline isomers, formed after unfolding, seems to not greatly inhibit the overall folding of the molecule but prevents close packing of structural elements into the highly cooperative, stable native form. The intermediates are very similar to the native protein in average structure but are much more motile.

J. Biochemical Lesions in Genetic Mucopolysaccharides.

1. The synthesis of methyl 2-deoxy-2-amino-α-D-glucopyranoside 4-sulfate completes the definitive synthesis of the three isomeric sulfate esters of α-methyl glucosaminide. This will permit the unambiguous assignment of sulfate esters in the glucosamine residues in heparin.

2. The 3,6 di-O-sulfated sulfamino methyl glucoside has been prepared and shown to be active as substrate for the partially purified 3-O sulfohydrolase. The product of the reaction is a mono-O-sulfated sulfamino glucoside which is not subject to further enzymatic desulfation. One may infer that this product is the sulfamino glucopyranoside 6-sulfate. The corresponding di-O-sulfated moiety in an oligosaccharide derived from heparin appears to be resistant to the enzyme. This unexpected discordance remains to be resolved.

K. Regulation of the Hormone-dependent Growth and Development of the Mammary Gland.

1. The interplay of insulin, glucocorticoid and prolactin induces the differentiation of the mouse mammary gland in culture. Earlier studies indicate that spermidine plays a key role in hormonal control of mammary development by mediating some of the lactogenic actions of glucocorticoid. In this study we examined the effect of progesterone, an inhibitor of lactogenesis, on milk-protein synthesis and spermidine accumulation. The maximal degree of inhibition, which was attained at 300 nM progesterone, was about 40% for casein synthesis, 90% for alpha-lactalbumin synthesis and 70% for spermidine accumulation. The increase in the activity of S-adenosylmethionine decarboxylase and spermidine synthase, two key enzymes in the polyamine biosynthesis, was inhibited by 55% and 65%, respectively, by progesterone. The extent of progesterone inhibition of milk-protein synthesis was greatest in cultured tissue from virgin mice, which contained a low level of spermidine whereas mammary explants from lactating mice, which contained a high level of spermidine, were insensitive to progesterone. The inhibitory effect of progesterone can be partially antagonized by cortisol which causes an enhancement of spermidine accumulation. These results suggest that the synthesis and accumulation of spermidine serve as a target site for the inhibitory action of progesterone on lactogenesis.

L. The Role of Free Inositol in Brain, Liver and Reproductive Organs.

1. In testis, inositol 1-phosphate synthase activity, shown previously to be reduced by hypophysectomy, was restored completely by testosterone and partially by gonadotropins. Diethylstilbestrol blocked the gonadotropins but not testosterone. Testosterone had no effect on synthase in intact animals. We conclude that testosterone is the immediate regulator of synthase activity in the testis. In the liver, synthase activity previously shown to be stimulated 30-fold by T_3 in hypophysectomized animals was found to be stimulated 3-fold in intact animals by T_3 and T_4. The increase was blocked by cycloheximide suggesting that protein synthesis accounts for the increase in activity. In streptozotocin diabetes,

synthase was also decreased in the liver. T_3 restored the activity to normal but insulin, alone, or with T_3 had no effect. We conclude that thyroid hormones are the immediate regulators of synthase activity in the liver.

2. Free inositol, derived from inositol 1-phosphate by a specific phosphatase present in excess in all tissues, was increased in testis by hypophysectomy and reduced to normal by both gonadotropins and testosterone. In the diabetic liver free inositol remained unchanged. Since the phosphatase was not subject to hormonal regulation free inositol synthesis is also regulated by controls on synthase. Differential utilization of inositol in liver and testis must then account for the differential response of the free inositol pools in those organs.

M. Studies on the Pathogenesis of Cystinosis.

1. Measurements of the rate of loss of cystine from lysosomes "loaded" as described above have established that the rate of such loss from cystinotic lysosomes is greatly retarded in comparison with the rate of escape from lysosomes of normal individuals; the corresponding rate of loss from lysosomes derived from individuals heterozygous for cystinosis was found to be intermediate between the two values. Other amino acids (e.g., methionine, tryptophan, leucine) exhibited equal rates of egress from similarly loaded normal and cystinotic lysosomes. Preliminary studies have also indicated that the rate of clearance of cystine from intact cultured fibroblasts derived from cystinotic and I-cell patients is significantly less than that observed in normal fibroblasts hyper-loaded with cystine by means of the ester technique.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 17001-16 LBM

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Mechanism of Hepatic Endocytosis of Serum Glycoproteins

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	Clifford Steer	Staff Fellow	LBM	NIADDK
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COOPERATING UNITS (if any)
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SECTION
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INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 5	PROFESSIONAL: 5	OTHER: -

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The process of asialoglycoprotein endocytosis by freshly isolated rat hepatocytes has been resolved into a series of experimentally defined parameters which have been examined by compartmental analysis and computer modeling. A monoclonal antibody directed toward the functional binding site of the hepatic receptor for asialoglycoproteins has been prepared. This reagent has served to provide evidence that all three subunits revealed by SDS-PAGE possess a unique determinant in common.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 17002-12 LBM

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Enzymatic Basis of Detoxication

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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TOTAL MANYEARS: 4	PROFESSIONAL: 3	OTHER: 1.0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

One means of detoxication for compounds foreign to the organism is their conversion to a mercapturic acid. Compounds bearing a highly electrophilic carbon atom react with glutathione to form a thioether of glutathione that is subsequently shorn of glycine and glutamic acid, yielding a thioether of cysteine. This is a key intermediate which can go on to form a mercapturic acid, i.e., the N-acetyl derivative of the cysteine thioether, as catalyzed by cysteine S-conjugate N-acetyltransferase in the presence of acetyl-CoA. However, a shunt mechanism exists in which cysteine conjugate β-lyase catalyzes the formation of the appropriate thiol, pyruvate and ammonia from the cysteine thioether; the thiol is detoxified by action of thiol S-methyltransferase. The three enzymes are under study.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 17003-15 LBM

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Polysaccharides in Morphogenesis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	Elena Mattia	Guest Worker	LBM NIADDK
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.0	4.0	1.0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Chitin shows a random distribution in the cell wall of several Saccharomyces cerevisiae mutants blocked in the cell cycle at a nonpermissive temperature. Chitin synthetase zymogen is transformed into active enzyme in proteinase B-defective yeast strains treated with digitonin. The activation process is inhibited by certain protease inhibitors and by chelating agents. Calcium ions reverse the effect of the latter.

A chitinase was purified from the growth medium of Serratia marcescens and used for the determination of chitin.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 17004-14 LBM

PERIOD COVERED
October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)
Thermodynamic and Kinetic Studies of Protein Structure and Enzymic Mechanisms

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Peter McPhie	Research Chemist	NIADDK, LBM

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None

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INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Our work is directed towards the relationship of protein sequence to conformation and enzymic activity. The digestive enzyme, pepsin, is synthesized as an inactive precursor pepsinogen. We are interested in the molecular basis of the proteolytic activity of the enzyme, how this is supporessed in the zymogen and is induced during activation.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 17005-17 LBM

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Studies of Naturally Occurring Sulfur Nucleotides: Identification, Chemistry, Biosynthesis and Biological Significance

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Marie N. Lipsett	Research Chemist	LBM NIADDK

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TOTAL MANYEARS: 1	PROFESSIONAL: 1	OTHER: -

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Project Terminated.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 17006-08 LBM

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Function of Bacterial Outer Membrane Components

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	John D. Foulds	Research Chemist	LBM NIADDK
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COOPERATING UNITS (if any)
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John Robbins, NIH, Bureau of Biologics
Hiroshi Nikaido, U. of California, Berkeley

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TOTAL MANYEARS: 5.5	PROFESSIONAL: 1.5	OTHER: 4

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Protein K is an Escherichia coli outer membrane protein found associated with the K1 capsule (a capsule associated with pathogenic strains). We have cloned the structural gene for protein K, purified the protein, demonstrated that it functions as a pore-forming molecule and examined the genetic control of its synthesis. We have also shown that any one of four distinct porin proteins is required for biosynthesis of the E. coli K1 capsule and that capsule biosynthesis in a porin-negative strain leads to the death of the cell.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 17007-01 LBM

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Intracellular Sorting and Movement of Proteins

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Richard Klausner	Medical Officer	NIADDK LBM
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	Krishnamurty Rao	Visiting Fellow	NIADDK LBM
	Ignacio Sandoval	Visiting Scientist	NIADDK LBM

COOPERATING UNITS (if any)

Jos Van Renswoude	Visiting Fellow	NCI, LTB
Matthew Pincus	Expert	NCI, LTB

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The detailed mechanism of iron uptake by human leukemic K562 cells were studied. These studies have documented a unique endocytotic pathway in which iron is carried into the cell attached to transferrin via the surface receptor for this serum glycoprotein. An acidic organelle was identified which is likely the site of iron transfer to cytosolic ferritin. At no point in this pathway does transferrin enter lysosomes but, rather, releases its iron and is then is itself released from the cell.

The unusual folding properties of ovalbumin were studied and the amino terminus was identified as the functional leader sequence.

Currently the glycoproteins of Herpes Simplex Virus are being studied with two major goals: (a) to study intracellular movement of the membrane glycoproteins and (b) to establish clonal cells lines that have integrated and express Herpes membrane glycoproteins in order to study the regulation of metabolism and topology of proteins in a living cell.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 18000-17 LBM

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Hormone-Dependent Development of Mammary Gland

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	Kevin Nicholas	Visiting Associate	LBM	NIADDK
	Jerzy Kulski	Visiting Fellow	LBM	NIADDK

COOPERATING UNITS (if any)
Project No. Z01 CB 08218-07
National Cancer Institute P.K. Qasba Expert
P. Chomczynski Visiting Scientist

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.5	4.0	.5

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Heretofore, insulin has been considered to be a metabolic and growth hormone. Our recent studies indicate that insulin is essential for milk-protein gene expression in mouse and rat mammary epithelial cells. The hormone is effective in vitro, in this respect, at physiological concentration, no other known factor can substitute for it, and its efficacy is independent of any influence on cell maintenance. Other reports suggest that insulin may also play a vital role in the development of certain other cell types. We propose that, in addition to being a metabolic and growth factor, insulin may also serve as a "developmental" hormone which is essential for selective gene expression in certain cells at particular stages of ontogeny.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 18001-08 LBM

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
The Role of Free Inositol in Brain, Liver, and Reproductive Organs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Frank Eisenberg, Jr.	Research Chemist	LBM	NIADDK
Other:	Ranganathan Parthasarathy	Visiting Fellow	LBM	NIADDK

COOPERATING UNITS (if any)
NONE

LAB/BRANCH
Laboratory of Biochemistry and Metabolism

SECTION
Section on Intermediary Metabolism

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	2.0	.5

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Studies of the hormonal regulation of inositol 1-phosphate synthase (EC5.5.1.4) in liver and testis have been extended. In testis, synthase activity, previously shown to be reduced after hypophysectomy, was partially restored by gonadotropins and completely by testosterone. Diethylstilbestrol blocked the gonadotropins but not testosterone. Testosterone had no effect on synthase in intact animals. We conclude that testosterone is the immediate regulator of synthase activity in testis. In liver, synthase activity was stimulated by T_3 and T_4 in the intact animal. Synthase activity, reduced in diabetic liver, was restored to normal by T_3 and T_4. We confirm our previous conclusion that thyroid hormones are the immediate regulators of synthase activity in the liver. Free inositol, derived from inositol 1-phosphate by a specific phosphatase present in excess in all tissues, was increased in testis by hypophysectomy and reduced to normal by both gonadotropins and testosterone. In the diabetic liver free inositol remained unchanged. Since the phosphatase was not subject to hormonal regulation, free inositol synthesis is also regulated by controls on synthase. Differential utilization of inositol in liver and testis must then account for the differential response of the inositol pools in those organs.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 18002-09 LBM

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Studies on the Pathogenesis of Cystinosis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Frank Tietze	Research Chemist	LBM NIADDK

COOPERATING UNITS (if any)
Dr. Joseph D. Schulman: Section on Human Biochemical and Developmental Genetics, NPMD, NICHHD.

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NIADDK, NIH, Bethesda, Maryland 20205

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Cystinosis is an inherited human disorder characterized by the excessive intralysosomal accumulation of the amino acid cystine. Although it has been suspected for some time that this disorder is due to a defect in the rate of passage of cystine across the lysosomal membrane, confirmation of this hypothesis has hitherto not been possible because of the lack of the experimental techniques necessary for relevant measurements. In the present investigation we have exploited a recently published methodology (J.P. Reeves, J. Biol. Chem., 254, 8914 [1979]) which makes possible the "loading" of human lysosomes with radioactive amino acids and have established that a probable defect exists in the passage of cystine across the lysosomal membrane of leucocytes from cystinotic patients.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 18003-09 LBM

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Regulation of the Hormone-dependent Growth and Development of the Mammary Gland

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Takami Oka	Research Chemist	LBM	NIADDK
OTHERS:	Kiyoto Motojima	Visiting Fellow	LBM	NIADDK
	Yuji Taketani	Visiting Fellow	LBM	NIADDK
	Hiroshi Wakimoto	Visiting Fellow	LBM	NIADDK
	Lisa J. Leiderman	Guest Worker	LBM	NIADDK
	Shizutoshi Nakagawa	Guest Worker	LBM	NIADDK
	John W. Perry	Research Assistant	LBM	NIADDK

COOPERATING UNITS (if any)

Dr. Criss, W.E. Howard University Cancer Center, Washington, D.C.

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Laboratory of Biochemistry and Metabolism

SECTION
Section on Intermediary Metabolism

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
7.5	5.0	2.5

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The interplay of insulin, cortisol and prolactin induces the differentiation of the mouse mammary gland in culture. Addition of progesterone (Prog) above 30 nM caused progressive inhibition of milk-protein synthesis and spermidine accumulation. The maximal degree of inhibition, which was attained at 300 nM, was about 40% for casein synthesis, 90% for α-lactalbumin synthesis and 70% for spermidine accumulation. The increase in the activity of S-adenosylmethionine decarboxylase and spermidine synthase, two key enzymes in polyamine biosynthesis, was inhibited by 55% and 65%, respectively, by Prog. The extent of Prog inhibition of milk-protein synthesis was greatest in cultured tissue from virgin mice, which contained a low level of spermidine, whereas mammary explants from lactating mice, which contained a high level of spermidine, were insensitive to Prog. The inhibitory effect of Prog can be partially antagonized by cortisol which causes an enhancement of spermidine accumulation that, in turn, mediates some action of cortisol on milk-protein synthesis. These results suggest that the synthesis and accumulation of spermidine serve as a target site for the inhibitory action of Prog on lactogenesis.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AM 18004-09 LBM

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Biochemical Lesions in Genetic Mucopolysaccharidoses

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Irwin G. Leder	Research Chemist	LBM NIADDK

COOPERATING UNITS (if any)

Ulf Lindahl, Department of Medical and Physiological Chemistry, The Biomedical Center, Uppsala, Sweden

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NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A novel sulfatase, specific for the 3-O sulfate ester of alpha methyl sulfamino glucopyranoside, has been partially purified from human urine. Using the enzyme as a structural probe, unique 3-O sulfated glucosamine residues have been located within an octasaccharide fragment of heparin having high affinity for antithrombin. All three isomeric sulfate esters of alpha methyl glucosaminide have now been prepared to provide standards for the identification of sulfated residues in heparin and to define the specificity of the enzyme. In the simple sulfated glucosaminide derivatives, the enzyme is not significantly inhibited by the presence of an additional sulfate group on the 6 position.

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PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Differentiation of Lymphoid Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Milton Kern	Research Chemist	LBM	NIADDK
OTHER:	Sastry Gollapudi	Visiting Associate	LBM	NIADDK
	Madduri Ramanadham	Visiting Fellow	LBM	NIADDK
	Blossom Johnson	Laboratory Assistant	LBM	NIADDK

COOPERATING UNITS (if any)
Dr. Daniel H. Zimmerman, Electronucleonics Laboratories,
Bethesda, Maryland 20014

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NIADDK, NIH, Bethesda, MD 20205

TOTAL MANYEARS: 4	PROFESSIONAL: 3	OTHER: 1.0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

It has been demonstrated that chloroquine inhibits the presentation of soluble antigen by accessory cells without affecting antigen binding by such cells. When added subsequent to a few hours incubation with antigen, chloroquine was not inhibitory. Since chloroquine is known to exert its inhibition of cellular function by alkalinization of vesicles that normally become acidified during the process of endocytosis, the present results may indicate that "processing" of soluble antigens by accessory cells may be required in addition to simple antigen presentation to T cells.

B cell heterogeneity among those cells capable of being activated by anti-immunoglobulin antibody has been shown by establishing the physical and functional non-identity of such B cells from peripheral blood on the one hand and spleen on the other.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 18006-11 LBM

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Enzymes of Carbohydrate Metabolism in Invertebrates and Vertebrates

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Marjorie R. Stetten	Research Chemist	LBM	NIADDK
OTHER:	Paul K. Goldsmith	Research Biologist	LBM	NIADDK

COOPERATING UNITS (if any)
NONE

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Laboratory of Biochemistry and Metabolism

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Section on Intermediary Metabolism

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	2.0	.5

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

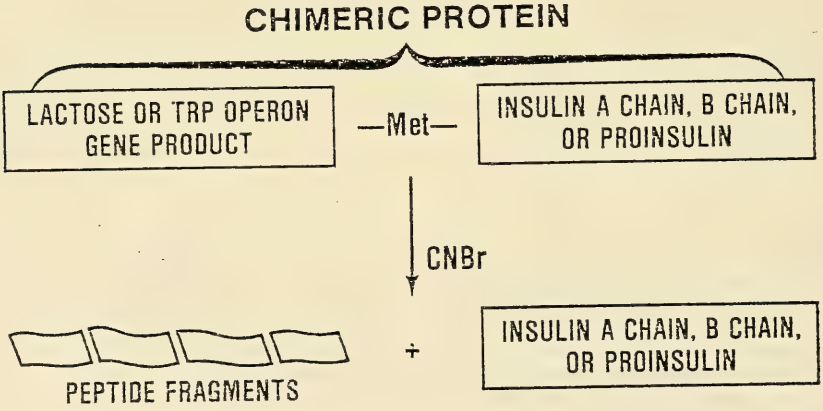
SUMMARY OF WORK (200 words or less - underline keywords)

Some of the pathways of utilization of blood glucose and its incorporation into tissue glycogen in Limulus polyphemus have been studied. Preferential loss of C-1 from glucose in vivo prior to incorporation into glycogen indicates a significant occurrence of the oxidative pathway of glucose metabolism in Limulus. Glucose is more rapidly and extensively incorporated into glycogen of muscle than into that of hepatopancreas, indicative of the relative importance of muscle in metabolic turnover in Limulus. Little gluconeogenesis from lactate or pyruvate was observed. Although glucose occurs at levels of only 3-5 mg per 100 ml of blood in Limulus, it is rapidly metabolized, about 80% of the ^{14}C of blood occurring as anions within 1 hour after administration of glucose-^{14}C. As much as 70% of Tritium administered on C-6 of glucose is excreted as $^{3}H_2O$ within 3 hours, showing an extensive, rapid glycolytic degradation of glucose in Limulus.

The Laboratory of Chemistry 1981-1982 at a Glance

The cyanogen bromide cleavage reaction has been used widely in protein chemistry since Witkop and the late Erhard Gross (1962) discovered that methionyl peptide bonds in proteins are cleaved in a highly specific manner by CNBr. Structural analyses of hundreds of proteins have been facilitated through use of this selective nonenzymatic cleavage. This discovery, which has been important in the laboratory for 20 years, has achieved a new application at Eli Lilly and Company as a key step in the large-scale manufacture of human insulin of recombinant DNA origin.

The present application is an extension of the small scale synthesis originally described by Goeddel *et al.* (1979) and further developed by Chance *et al.* (1981) for human insulin. By means of recombinant DNA technology the gene for insulin A chain, B chain, or proinsulin is linked via a methionine codon to a portion of either the lactose or tryptophan operon, which codes for proteins normally produced by *E. coli* in large amounts. This synthetic gene is inserted into the plasmid of *E. coli* and the bacterium expressed many copies of the new chimeric protein:


CHIMERIC PROTEIN
LACTOSE OR TRP OPERON GENE PRODUCT
—Met—
INSULIN A CHAIN, B CHAIN, OR PROINSULIN
CNBr
PEPTIDE FRAGMENTS
+
INSULIN A CHAIN, B CHAIN, OR PROINSULIN

Either insulin A or B chain or proinsulin is then released in high yield by means of the CNBr cleavage. After appropriate purification and conversion of these insulin components, crystalline human insulin is obtained for the treatment of diabetes.

Twenty Years of Research on Novel Frog Toxins

Just twenty years ago Fritz Märki, Fellow in the Visiting Program from Switzerland, isolated Batrachotoxin. Many more toxins have then been studied subsequently in an interdisciplinary research effort which has served as a model for the proper approach to the chemistry of Natural Products in a biomedical environment. These two decades have now been reviewed for Volume XXI of Manske's The Alkaloids (ed. Arnold Brossi).

"Remarkable progress toward a practical synthesis of codeine--Discovery of novel and highly active morphinan analgesics" is the title of a progress report by Arnold Brossi in TIPS (Trends in Pharmacological Sciences, June 1982). Outlook and consequences of these discoveries form part of the subsequent sectional report.

The "tenuous bridge between the two worlds of muscarinic and nicotinic acetylcholine receptors", *viz.* acetylcholine, has been widened: arecoline, the classical muscarinic agonist of John Newport Langley, has been modified to arecolone methiodide, nicotinic agonist eight times stronger than acetylcholine. This program of new agonists has now been raised to the level of computer modeling both for ligands and receptors.

New Developments in Fluorine Analogs of Metabolites, Neurotransmitters and Hormones

The synthesis of the long-sought antimetabolite 2-fluoro-4-aminoimidazole-5-carboxamide has eeen achieved after considerable effort; this compound is expected to be a potent antiviral agent and affinity label for enzymes involved in purine biosynthesis.

Analogs of the hormone TRF containing fluoro and trifluoromethyl groups have been synthesized; several of these analogs may have greater potency for prolactin release than the natural peptide.

The first synthesis of a hydroxylaminoimidazole has been achieved by displacement of fluorine with hydroxylamine. These compounds are suspected to be the true cytotoxic agents generated in the metabolism of nitroimidazoles.

Studies on the pharmacology of fluorinated biogenic amines have led to a new theory on the recognition and binding of aromatic rings by enzymes and receptors; this theory holds that the protein can recognize the differences in electron desntiy at each ring carbon, and that such differences control the orientation of the ring in the binding site.

Progress on Interferon and Its Mechanism of Action

Systematic exploration of the relationship of oligonucleotide structure to biological activity has produced an analogue of the unique molecule *2',5'-Oligoadenylate (2-5A)*. This new analogue is, unlike its parent, *extremely* resistant to degradation by the phosphodiesterase activities of cells and is ten times more potent than the naturally-occurring 2-5A as an inhibitor of translation with only 10^{-10} M sufficient to cause a 50% inhibition of protein synthesis.

In a collaborative venture with Dr. H. Sawai of the University of Tokyo, it has been demonstrated through an unambiguous synthesis of a cordycepin (3'-deoxyadenosine) analogue of 2-5A, that the 3'-hydroxyl groups of 2-5A are not needed for binding to the 2-5A activated endonuclease, but are required for activation of the enzyme.

A rapid enzyme-linked immunoassay for 2-5A, developed in this laboratory, has been employed to isolate the first monoclonal antibodies to 2-5A.

On an international level the Laboratory has received a commitment from the President, Deutsche Forschunpsgemeinschaft (German Research Association) for financial support of Guest Workers from, or cooperative research projects with, academic Institutions or Max Planck Laboratories in the Federal Republic of Germany. Applications are pending.

Past and present achievements of the Laboratory have been honored by Sweden, the Federal Republic of Germany and Japan by invitations to Special Lectures or honorary memberships in learned societies.

SECTION ON BIOCHEMICAL MECHANISMS

Chemistry of Imidazoles

2-Trifluoromethyl-L-histidine and 2-trifluoromethylhistamine are readily obtained from L-histidine and histamine, respectively, by a novel three-step route (see earlier reports). These compounds are of considerable biological interest because they show the unique property of losing hydrogen fluoride above pH 9 to form metastable difluorodiazafulvenes. The latter species have the potential to serve as covalent affinity labels for imidazole recognition sites in enzymes and receptors, particularly *in vivo*. Since the loss of hydrogen fluoride is very slow at physiological pH, we have been exploring methods to increase reactivity by introduction of additional substituents into the imidazole ring. Two electronic effects are necessary: (1) an electro-withdrawing group which will enhance the acidity of the ring-NH and thus facilitate loss of hydrogen fluoride at lower pH; unfortunately, such a group also decreases the reactivity of the imidazolate anion enormously; (2) a group with lone-pair electrons capable of stabilizing the fulvene intermediate by resonance with the ring and, thus, of reducing the activation energy necessary for loss of fluoride ion. We have prepared and studied a variety of 2-trifluoromethylimidazoles with additional substituents. A computer analysis of the kinetics of hydrolysis of the trifluoromethyl group provided a linear correlation between log k and the composite contributions of resonance and inductive effects. This correlation allows us to select an additional substituent to produce any desired reactivity range at a specified pH. Furthermore, our correlation analysis indicates fluorine to be the most logical extra substituent for the achievement of the desired electronic effects. This result is gratifying since fluorine is sterically the most acceptable replacement for hydrogen in a recognition site (because of its minimal size) and because we have, ourselves, developed the only known method for the synthesis of fluoroimidazoles (see below).

At the same time, we had found 4-trifluoromethylimidazoles to be about ten times as reactive as the 2-isomers. Unfortunately, our facile synthesis of the 2-trifluoromethyl series is not applicable to the 4-series, and the only known approach to the latter compounds would have required a laborious 15-step synthesis. We found, however, that the trifluoromethyl group can be introduced into the imidazole ring in *one step* by irradiation with trifluoromethyl iodide in methanol solution. Mixtures of the 2- and 4-isomers are obtained in high yield, and the isomers are readily separated by chromatography. The photochemical method can be used directly with histamine and histidine (and with other complex imidazoles), providing excellent yields of both isomers. Thus, both 2- and 4-trifluoromethyl- imidazoles can be obtained in a single synthetic step. Surprisingly, the photo- chemical method fails utterly with most other heterocyclic systems. A number of 4-trifluoromethylimidazoles have shown significant activity as therapeutic agents for gout and cardiac problems. Our discovery of the photochemical synthesis makes this series of compounds far more readily accessible and in much higher yields than the purely chemical route.

From published pharmacological data, it is evident that the trifluoromethyl group, despite its bulk, does not interfere with binding of drugs to certain recognition sites. In fact, our preliminary data have shown that both 2- and 4-trifluoromethylhistamine bind quite well to cyclic AMP phosphodiesterase, but not irreversibly. On the basis of our electronic predictions, we are now involved in the synthesis of the isomeric fluoro-trifluoromethylhistamines (photochemically), with the expectation that these analogs may bind *irreversibly* to the enzyme. In parallel work, analogs of TRF have been synthesized with both 2- and 4-trifluoromethyl groups on the imidazole ring of histidine. These compounds are now being evaluated for their ability to bind to TRF receptors, to promote prolactin release and, possibly, to form irreversible covalent attachments. Again, greater promise is expected for the fluoro-trifluoromethyl derivatives of histidine, and synthetic studies are in progress. Efforts to introduce the trifluoromethyl group photo- chemically into preformed histidine peptides have, thus far, been unsuccessful. [L. A. Cohen, H. Kimoto, S. Cohen, V. Labroo, R. Henkin, P. Dannies].

The valuable properties of nitroimidazoles as radiation sensitizers and as selective cytotoxic agents for cancer treatments have stimulated considerable research into mechanism of action and metabolic fate of the drugs. One theory holds that the nitroimidazole is reduced in vivo to the hydroxylaminoimidazole, which functions as a supernucleophile in cleaving phosphate ester bonds and in degrading polynucleotides. Thus, the electrochemical reduction of nitroimidazoles in the presence of DNA or RNA leads to degradation of the polynucleotides. Neither nitroimidazoles, themselves, nor aminoimidazoles show this catalytic property. It is presumed, therefore, that degradation is effected by transient reduction products, the hydroxylaminoimidazoles. Efforts in many laboratories to synthesize hydroxyl- aminoimidazoles have failed, since reduction of the nitro compounds leads rapidly to the amino analogs. Our discovery of a unique photochemical route to 2-fluoro- imidazoles makes available an alternative approach to the 2-hydroxylamino- imidazoles, since fluorine is the only C-2 substituent known to be capable of nucleophilic displacement. Thus, preliminary studies have shown that 1-methyl-2-fluoroimidazole does react readily with hydroxylamine and this reaction is being explored for its preparative possibilities. [L. A. Cohen, R. Breiman].

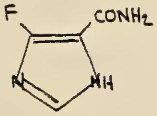
The facility with which 4-trifluoromethylhistamine and histidine can be prepared opens the way for a new route to the rare 2-substituted analogs of these metabolites. For example, 4-trifluoromethyl-L-histidine is converted, in alkaline methanol, to the corresponding carbomethoxy derivative. This product is brominated at C-2, the only available ring position. The product is hydrolyzed in aqueous acid, and the carboxyl group is lost simultaneously by decarboxylation to give 2-bromo-L-histidine. This use of trifluoromethyl as a temporary blocking group has far-reaching consequences for simplifying the syntheses of some complex imidazoles which are of clinical interest. [L. A. Cohen, V. Labroo].

The first synthesis of 2-iodo-DL-histidine has been achieved by a different route: 1-trityl-4-hydroxymethylimidazole is lithiated at C-2 and the product is reacted with N-iodosuccinimide. The resulting 2-iodo derivative is detritylated, converted to the 4-chloromethyl derivative, and alkylated with acetamidomalonic ester; hydrolysis and decarboxylation afford pure 2-iodo-DL-histidine. When the same route was attempted for the preparation of 2-bromohistidine, the hydromethylimidazole underwent extensive oxidation. The alternative approach, via trifluoromethylhistidine (described above), avoids problems of reactive halogenating agents and leads directly to the chiral isomer. The conversion of 2-aminohistidine to 2-azidohistidine is being completed; the latter compound, which should prove useful for photoaffinity labeling, has been found to be quite photosensitive in ordinary light and controlled breakdown may prove to be a problem. [L. A. Cohen, V. Labroo, R. Jerussi, K. L. Kirk].

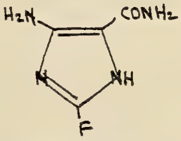
In continuation of our studies on nonaromatic tautomers of imidazoles, we have shown that 1-methyl-2-aminoimidazoles and 1-methyl-2-hydroxyimidazoles undergo facile reduction of the 4,5-double bond because of dearomatization. This observation accounts for some of the difficulty in obtaining pure samples of 2-aminohistidine following hydrogenation of the 2-arylazo precursor, the loss of 4-substituents during the reduction of other 2-arylazoimidazoles, and other puzzling phenomena. As further evidence of the small energy difference between imidazoles and their isoimidazole tautomers, we have shown that trifluoromethylimidazoles react with secondary amines to give relatively stable N, N, N',N'-tetrazafulvenes, with isomerization to imidazoles requiring as long as 24 hr. The unusual isotope exchange at C-4 of urocanic acid in the presence of urocanase, and prior to its conversion to imidazolonepropionic acid is also explained by this tautomerism. [L. A. Cohen, V. Labroo, R. Breiman].

Fluoroimidazoles

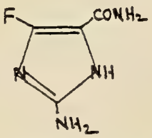
Our earlier studies had shown 4-fluoroimidazole-5-carboxamide riboside to be a potent antiviral agent in cell culture systems. Furthermore, it had been demonstrated that, in human blood, the aglycone undergoes ribosylation and the riboside is phosphorylated by ATP to a triphosphate. Since the final product could not continue along the pathway to form an adenine ring, the stage of the metabolic block seems to be well specified. We wished, therefore, to extend our studies to include the ribosides of 2,4-difluoroimidazole-5-carboxamide and of isomeric aminofluoroimidazoles. Fluoroimidazoles are obtained by photochemical replacement of amino groups, and aminoimidazoles are generally prepared by hydrogenolysis of arylazoimidazoles. Although the procedures work smoothly for a variety of imidazoles, the hydrogenolysis of an arylazoimidazole containing an additional fluoro or amino substituent results in the removal of the latter group. This obstacle to synthesis has been overcome by the application of a unique reducing agent, formamidine-2-sulfinic acid. Thus, 4-fluoro-2-aminoimidazole-5-carboxamide was prepared and was converted into the 2,4-difluoro derivative. Surprisingly, the isomeric 2-fluoro-4-aminoimidazoles are far less stable and must be stored at very low temperatures. Since these compounds may not survive further chemical manipulations, the ribosylation of the imidazoles by enzymatic means is being explored. 2,4-Difluoroimidazole-5-carboxylic ester was planned as a precursor to 2,4-difluoro- histidine. To date, every attempt to reduce the ester function to an alcohol has resulted in loss of fluorine. Alternative approaches are now being examined. [L. A. Cohen, Y. Takahashi, K. L. Kirk].



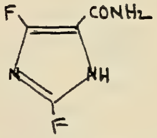
4-fluoroimidazole-5-carboxamide



4-aminoimidazole-5-carboxamide
2-fluoro



2-amino-4-fluoroimidazole-5-carboxamide

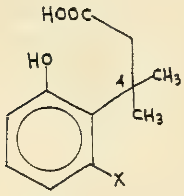


2,4-difluoroimidazole-5-carboxamide

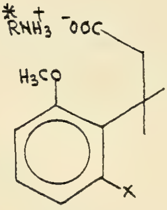
Some years ago, a sample of 4-fluoro-His-TRF was prepared and evaluated for us at the Salk Institute, and was found to have negligible activity in prolactin release assays. In 1981, Fromageot reported a synthesis of (allegedly) the same compound by introduction of fluorine into the preformed TRF. His material showed higher activity than TRF itself, but certain physical properties of the product were inconsistent with those we would have expected for the fluoro analog. We have now resynthesized both 4-fluoro-His-TRF and 2-fluoro-His-TRF from the authentic amino acids and are awaiting the results of prolactin release assay. The relative instability of 2-fluorohistidine in aqueous media required the development of modified procedures for peptide synthesis. We were surprised to find that the fluorine substituent in the amino acid is considerably more stable than that in peptides, opening new possibilities for use of the 2-fluoro group in affinity labeling. We have recently developed an analytical procedure to follow the rate of release of fluoride ion from these compounds at very low concentration and are now examining the effect of structure, charge and pH on the stabilities of 2-fluoro- imidazoles. [V. Labroo, K. L. Kirk, L. A. Cohen, P. Dannies, M. Troung].

Stereopopulation Control

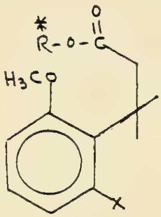
We have ascribed the very high rates of lactonization of A to the fact that the side-chain carboxyl group is held very close to the phenol by conformational freezing; the latter effect is achieved by interlocking between X and the methyl groups at C-4. The greater the size of X, the greater the barrier to rotation of the carboxyl chain out of its cisoid conformation. Indeed, earlier NMR studies and computer calculations have suggested that, when X = CH_3, the barrier is so great that free rotation is not possible even at 200 °C. Since the three-carbon side chain cannot be coplanar with the benzene ring, it follows that molecular chirality is present and resolution into enantiomers should be possible if the barrier to rotation of the side chain is really > 20 kcal per mole.



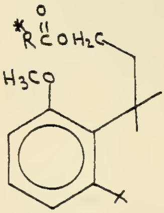
A



B



C



D

Stabilized derivatives of A have been prepared with chiral handles (B, C, D), thus producing potential diastereomers. Species B-D have proved resolvable, although the magnitude of optical activity due to the substrate is rather small. We are currently investigating the temperatures necessary to effect racemization of the diastereomers and the possibility of differentiation by NMR techniques. [L. A. Cohen, M. M. King, W. Antkowiak].

The van der Waals radius of X is linearly related to the steric component of the rate constant for lactonization of A. This result demonstrates the existence of a continuous free energy system which includes both small and large groups; the only previous demonstration of this phenomenon involved studies on the rates of racemization of chiral biphenyls. We have also found that the deshielding of ^{1}H NMR signals by van der Waals effects through space are linearly related to the size of the substituent, the first time that this poorly understood phenomenon has received quantitative treatment.

At pH 11, lactone A is hydrolyzed to phenolate ion. The latter species is unstable over a period of hours and generates the corresponding quinone. Our original assumption that this change was due to air oxidation is incorrect and it now appears that the reaction represents the first example of an ElcB elimination involving the reductive cleavage of an oxygen-sulfone bond. Thus, the product of this breakdown should be sulfinate ion, not sulfonate. This thesis is now under investigation by NMR techniques. [L. A. Cohen, M. M. King, M. Troung].

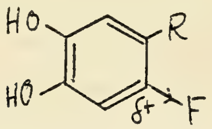
Chemistry and Biology of Fluorinated Biogenic Amines

We have reported the effect of fluorine substitution on the regioselectivity of the catechol O-methyl transferase (COMT)-catalyzed methylation of ring-fluorinated norepinephrines (FNE). The results clearly indicate that increasing phenol acidity increases the degree of methylation--suggesting that phenol ionization plays a role in transmethylation. These results were obtained by indirect measurements of methylated products--by converting them to fluorinated vanillins and isovanillins by periodate oxidation. With the goal of determining precisely the kinetic pKa's of ortho- and meta--methylations as well as the values of K_m and V_{max} for the reactions, we required a more convenient and more accurate assay system. To this end, HPLC separations of the normetanephrines and norpara- nephrines have been achieved. As a first step toward providing unambiguous product identification, 5-fluornormetanephrine and 5-fluoronorparanephrine have been synthesized from 5-fluorovanillin and 5-fluoroisovanillin, respectively. Syntheses of the corresponding 2-fluoro- and 6-fluoro derivatives are in progress.

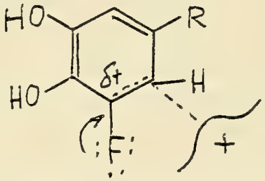
The pH rate profile for the degree of meta- and para-methylation of NE was obtained using the HPLC assay system. While the absolute amount of meta methylation (N 90% of total methylation at pH 7) remained essentially constant, the amount of para methylation increased with pH with an apparent pKa for the reaction of ∿ 8.5. These data could be interpreted as reflecting ionization of the para-hydroxy group or as ionization of a functional group at the enzyme active site. Results from a similar study with 5-FNE (having a lower phenolic pKa) should differentiate between these choices.

The enzymatic transmethylation kinetic constants are significantly affected by fluorine substitution. Thus, we, and others, have shown that substitution of fluorine in the 5-position of NE or DOPA results in an increased rate of methylation resulting from a decreased K_m (0.16 nM) and an increased V_{max} (37.7 nmol mg^{-1} min^{-1}) (Values for NE: $K_m = 0.24$, $V_{max} = 3.9$). This phenomenon has complicated the development of 5-^{18}F- DOPA as a positron-emitting scanning agent. In contrast, 6-FNE is a poorer substrate than NE for COMT, primarily as a result of its much poorer affinity for the enzyme's active site ($K_m = 4.7$ nM). This fact suggests that 6-F-DOPA and 6-F-α-methyl DOPA could offer advantages in the design of medicinal agents (cf α-methyl DOPA as an antihypertensive agent and L-DOPA in treatment of Parkinson's disease) and scanning agents, because of significantly greater biological half-lives compared to the 5-F- or the unsubstituted relatives. We have synthesized 6-F-DOPA and 6-F-α-methyl DOPA, and biological evaluations are in progress.

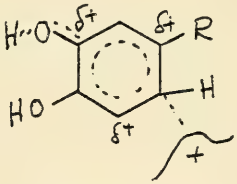
In addition to the practical aspects of the decreased affinity of 6-FNE for COMT, the mechanism for the effect of fluorine substitution on COMT kinetic constants may have relevance to the important question of the regioselectivity of COMT- catalyzed methylations. In parallel with our recent proposal for the mechanism of fluorine-induced specificities for α- and β-adrenergic receptors, the low affinity of 6-FNE for COMT is most readily explained by an inductive blocking of a bonding interaction between the 6-position of 6-FNE and an electrophilic center in the active site. This same bonding interaction of the unsubstituted catecholamine would effectively increase the acidity of the 3-hydroxyl (relative to the 4-hydroxyl) and thus explain the preference for meta-methylation. Similarly, fluorine in the 5-position should stabilize an electrophilic bonding interaction (since fluorine acts through resonance as an activating substituent for electro- philic aromatic substitution).



Fluorine at 6-position inductively decreases electron density at C-6, decreasing attraction to positive center on enzyme surface.



Through resonance, fluorine on C-5 stabilizes electrophilic interaction at C-6.



Electrophilic interaction at C-6 increases acidity of OH at C-3.

To evaluate this speculative scheme, careful HPLC analyses of the isomeric FNE's will be carried out with the goal of determining accurate meta-para ratios and kinetic constants as a function of pH. In addition, the syntheses of the three isomeric difluoro NE's are in progress to provide additional compounds as mechanistic probes. (D. Thakker and C. R. Creveling, LBC; K. Buchhold, G. Chen, R. Antkowiak and K. Kirk).

Pharmacological studies of 6-FNE and 2-FNE as specific adrenergic agonists have continued. Exogenously applied NE interacts with both alpha and beta adrenergic receptors in the rat hippocampus resulting in a decrease and an increase in excitability, respectively, as determined by selective alpha- and beta-adrenergic agonists and antagonists. Perfusion of rat hippocampal slices with 6-FNE and 2-FNE led to decreases and increases, respectively, in excitability, consistent with previous results, and confirming the selectivities of the FNE's. (B. Hoffer and T. Dunwiddie, U. of Colorado; K. Kirk).

Fluorinated Molecules as Biological Tracers

Previously, we had analyzed storage of 4,6-difluoro-5-hydroxytryptamine in human and pig platelets using ^{19}F-NMR. We now have extended this technique to what may be the first direct NMR observation of intravesicular catecholamines. The fluorine chemical shift (relative to C_6F_6) of 6-fluorodopamine is 36.5 ppm (1 mM in 0.01 N HCl) with a line width of 32 Hz. Incubation of 6-FDA with guinea pig striatal microsacs for 30 min at 37° resulted in a decrease in intensity and a broadening of the peak to 134 Hz. At the same time, peaks corresponding to 6-fluorodihydroxyphenylacetic acid (6-FDOPAC) (37.2 ppm) and an unidentified metabolite (44.5 ppm) appeared. Effects of drugs to specifically block vesicular uptake and/or metabolism were followed by NMR and demonstrated that the observed 6-FDA peak was indeed from vesicular 6-FDA. Concomitantly, uptake and metabolism were followed by HPLC. 6-FDOPAC was identified by comparison with an authentic sample synthesized unambiguously from 6-fluoroveratraldehyde. (J. Costa, C. C. Chiueh, NIMH; E. Sokoloski, NHLBF; D. Diffley, Johns Hopkins; and C. R. Creveling, LBC; and K. Kirk).

SECTION ON CARBOHYDRATES

Monoclonal Anti-Galactan Immunoglobulins

Following the elaboration of a computer model for monoclonal IgA J539, binding studies were continued and it was confirmed that the immunoglobulin has a groove-type combining site. Also, an affinity-label was synthesized, and it was shown that anti-galactan IgA could be affinity labeled in the combining site.

The Serological Response to Cryptococcus Neoformans

Rabbit immune sera to *C. neoformans* Type D were obtained, and the antibodies were fractionated by affinity chromatography. Using computer models of the antigen (constructed by Hard Sphere Anomeric Effect Calculations) it could be shown that the antibodies appear to be directed mostly towards the terminal groups on the Type D antigen and that the backbone of the antigen has no immunodeterminants.

Antiheparin and Anti-DNA Monoclonal Immunoglobulin

The purification and characterization of a monoclonal IgG which reacts with heparin, DNA and other negatively charged antigens has been described. This monoclonal antibody may be useful in the identification of these antigens on cell surfaces.

SECTION ON MEDICINAL CHEMISTRY

Relationship of Benzomorphan Analgesics to Dissociative Anesthetics.

An X-ray crystallographic study of dexoxadrol, a dissociative anesthetic known to interact with phencyclidine binding sites obtained from mammalian brain, was used to determine possible points of overlap with benzomorphans which also interact with the PCP binding sites. This effort to delineate the relationships between the structures of apparently diverse molecules which have phencyclidine-like activity in vivo and also interact with phencyclidine binding sites in vitro, resulted in the synthesis of "analgesic" compounds with three-point overlap, in three-dimensional space, to specific moieties in dexoxadrol. The examination of these compounds is being actively pursued. (A. E. Jacobson, B. S. Bajwa, K. C. Rice, M. Rafferty, J. V. Silverton, and J. H. Woods).

Evaluation of The Dependence Potential and Abuse Liability of Antinociceptives

Over 100 compounds, obtained from our own Laboratory and from universities and industrial groups in the US, Europe and Asia, were examined to determine their antinociceptive activity in various rodent assays. In collaboration with the Departments of Pharmacology of the University of Michigan Medical School and the Medical College of Virginia, under the auspices of the Committee on Problems of Drug Dependence, some of these compounds were further examined to determine their dependence potential and abuse liability. Data obtained from these studies has served to alert individuals about possible side-effects of compounds before clinical introduction of the drug, and to focus attention on those drugs which might serve as specific probes for subpopulations of opiate receptors. (A. E. Jacobson, E. L. Atwell, J. H. Woods, J. L. Katz, F. Medzihradsky, C. B. Smith, L. S. Harris, M. D. Aceto, E. L. May, and G. D. Winger).

Chemical-Biological Features of Colchicine and Congeners.

The colchicine structure has been further scanned in order to locate the sites which are most critical for the antimitotic effect and for the noted toxicity. It has now been established that the 10-OMe group is not as critical as assumed, and that the substitution in the aromatic ring and the nature of the acetamido side chain may be more important. A large series of novel N-acyldeacetylcolchicines has been prepared here and at the Istituto Superiore di Sanita in Rome. Larger quantities of the 1,2- and 2,3-catechols have been prepared for testing at the NCI. The synthesis of the N-sinapinoyldeacetylcolchicine, possibly representing a photoaffinity label, and the synthesis of the ester of colchifoline with 2,2,5,5-tetramethyl-3-pyrrolin-1-yloxy carboxylic acid and having a spin label, has been accomplished and their binding to tubulin will be investigated at the Laboatory of Environmental Biophysics at Research Triangle Park. Additional quantities of unnatural 1,2-methylenedioxy-1,2-didemethylcolchicine, which is distinctly less toxic than colchicine or its natural 2,3-isomer have been prepared. (P. Sharma, A. E. Jacobson, L. Atwell, M. A. Iorio, C. F. Chignell and A. Brossi).

Progress Towards a Synthesis of Natural Toxins.

The synthesis of natural anatoxin-a, a substance from the blue algae, has been repeated from cocaine by the Edwards approach, affording besides the natural toxin several analogs needed for testing in the nicotinic acetylcholine receptor assay. Its total synthesis from 2-(5'-oxohexyl)-pyrrolidin-2-one prepared by novel methodology has thus far been unsuccessful. The 3-n-propylpyrrolizidin-5-ones (E and Z) served as starting materials to prepare 5-methylated model compounds of defined stereochemistry, needed to guide the synthesis program of natural ant toxins belonging to this class of compounds. (K. Takahashi, P. Buchs, B. Witkop and A. Brossi).

Semirigid and Rigid Conformers of THP.

The question whether tetrahydropapaveroline (THP) can be modified to afford agents which are more selective in their binding to the adrenergic and dopaminergic receptors was further investigated. Many novel representatives having different aromatic oxygenation have been prepared, including aromatic isoquinolines and hexahydrobases. Several representatives of the benzocycloheptisoquinoline family have been prepared from the berbine alkaloid coreximine by fission of the $N-C_8$ bond with chloroformates. The benzyl halide intermediates could also be catalytically reduced to 6'-methyl-substituted norreticuline derivatives and converted into 6'-methyl-substituted THP. A somewhat greater selectively has earlier been observed with 6'-bromo THP and the speculation, that the introduction of a 6'-bromo substituent might either block a metabolically active position or introduce sufficiant bulk to stabilize a particular conformation of THP, is now being tested with the 6'-methyl analog. The introduction of larger alkyl groups by the route chosen is planed. (P. Sharma, F.L. Hsu, H. Schmidhammer, P. Buchs, K. C. Rice, P. Skolnick and A. Brossi).

Problems with Antifolates.

The antimalarial dihydrotriazin WR-99210 is active against Plasmodium berghei strains resistant to cycloguanil and pyrimethamine. The presence of a masked hydroxylamine function and a 2,4,5-trichlorophenoxy substituent makes WR-99210, together with its poor absorption and its poor stability around pH 7 an interesting compound for further study. It has now been found that WR-99210 and similar oxydihydrotriazines decompose, when refluxed with 1N HCl, affording an oxime ether part and a C_2N_4-fragment not yet isolated. The oxime ether derivative could be further manipulated and chemically pure biguanides and oxydihydrotriazines could be obtained, needed for antimalarial testing at Walter Reed.

The X-ray data obtained with several aromatic tetramethoxy-substituted tetrahydroisoquinoline Cactus alkaloids revealed that all the methyl groups sitting on the aromatic oxygens were out of the plane of the aromatic ring. This prompted a synthesis of tetramethoprim and pentamethoprim, two higher methoxylated analogs of the antibacterial trimethoprim, and prepared from the corresponding benzaldehydes by published procedures. A comparision of tetramethoprim with trimethoprim against a variety of yeast and bacteria in vitro revealed that the former was inactive, whereas the latter was highly active. It is thus firmly established that the aromatic substitution in these compounds is critical, and that the presence of 3,4,5-trimethoxy system obviously provides a situation which is needed for a fit into a receptor site. (B. Bajwa, K. Takahashi and A. Brossi).

The 6-Ketomorphinan Story.

The ketomorphinans represent an interesting bridge between natural and synthetic opioids and are also valuable models for studying the importance of aromatic substitution with regard to antinociception. Such a study has now been completed with the synthesis and biological evaluation of all four possible mono-phenols and their O-methyl ethers. The availability of 2-oxygenated compounds made it, furthermore, possible to prepare the aromatic unsubstituted ketomorphinan, which when tested in the hot plate-assay as its N-methyl derivative showed unusually high antinociception. Whereas the 2-oxygenated series became available by a classical Grewe cyclization, the 1-oxygenated series had to be constructed from its 4-hydroxy isomer prepared from natural morphine. The position of the oxygen function in the aromatic ring is crucial for antinociception, and this study represents a marvelous example of how little one can really predict based on existing knowledge. The 2- and 1-oxygenated N-methylketomorphinans are practically inactive as antinociceptive agents, whereas the 3- and 4-oxygenated isomers are highly potent. The nature of the oxygen function is also important and the rules are quite different in the morphinan and in the ketomorphinan series. In the former, the phenols are more active than their O-methyl ethers, whereas the latter are more potent in the 6-ketomorphinan series. Substitution at the N-function, and at C-14 with hydroxy, gave the expected information, previously experienced in the morphinan series. Since 6-ketomorphinans can readily be deoxygenated to afford corresponding morphinans, and also modified at the carbonyl group, they represent more versatile and possibly more interesting compounds than their morphinan relatives. (H. Schmidhammer, A. E. Jacobson, L. Atwell and A. Brossi).

Total Synthesis of Natural and Unnatural Opioids.

The total synthesis of (±)-3-deoxy-7,8-dihydromorphine has been imporved. The chemistry of by-products obtained in critical steps was solved. The synthesis of (±)-4-methoxy-N-methylmorphinan-6-one has also been accomplished and is now is such shape that it can easily be adopted to the preparation of (+)-enantiomers, needed for the evaluation of their antitussive effects. (F.L. Hsu, H. Schmidhammer, K. C. Rice and A. Brossi).

Further refinements have been made in the previously described total synthesis of either enantiomer dihydrocodeinone, which is convertable by established, highly efficient methodology to codeine, morphine and thebaine. The overall yield of the latter three compounds from m-methoxyphenethylamine is in the range of 15-20%. Since the entire spectrum of medically valuable opiates consists of codeine, morphine their transformation products, and the drugs based on thebaine, either the natural or unnatural enantiomer of this group of drugs is available by total synthesis. Preparation of selected unnatural opiate enantiomers is in progress at Research Triangle Institute under the auspices of NIDA. (K. C. Rice)

Probes for Study of the Mechanism of Action of Phencyclidine, Tricyclic Antidepressant, and Opiate Receptors.

The enantiomers of the 3-methyl derivative of achiral phencyclidine have been shown to stereospecifically displace specifically bound [^{3}H]-phencyclidine rat olefactory bulb slices, confirming the degree of stereospecificity observed for these enantiomers in several other behavioral and electrophysiological paradigms. The utility of these enantiomers in further characterization of phencyclidine receptors in other systems is under investigation. Several nitroimipramine derivatives have been shown to bind essentially irreversibly at 0°C to high affinity binding sites for imipramine. Since this binding is reversible at 37°C, these derivatives may well prove to be of value in isolation of the binding sites when incorporated into an affinity chromatography system. Synthetic work toward synthesis of specific, irreversible inhibitors for subpopulations of opiate receptors, has recently been initiated in an attempt to deliniate the functional significance of opiate receptor subpopulations. Preliminary biological results indicate irreversible ligands have been obtained which are specific for the mu and delta subpopulations. Further synthetic work and determination of the utility of these ligands in purification and characterization of opiate receptors is being vigorously pursued. (K. C. Rice, A. E. Jacobson, T. Burke, B. Bajwa and M. Rafferty).

Isolation and Characterization of the Antitrypanosomal factor from Pseudomonas Fluorscins.

Chromatographic purification of extracts of *Pseudomonas fluorscins* has afforded several fractions which are active *in vivo* (mice) and *in vitro* against *Trypanosoma cruzi*, the pathogenic cause of South American sleeping sickness. Final purification of these active fractions using gel filtration and High Pressure Liquid Chromatography is in progress (K. C. Rice and M. Rafferty).

Synthesis and Pharmacology of Irreversible Inhibitors and other Neuropharmacological Probes of Benzodiazepine Receptors.

A relatively clear picture of the structure-activity relationship in the β-carboline area has been obtained by a comprehensive study in *in vivo* and *in vitro* systems. Several β-carbolines have been synthesized and identified as benzodiazepine antagonists. Other β-carbolines examined in this study are being utilized to provide additional information on the benzodiazepine receptor complex (K. C. Rice).

Double-Stranded RNA and 2',5'-Oligoadenylate: Companions in Interferon Action?

Analogs of dsRNA and of 2-5A and its derivatives can provide the means to dissect the relative importance of the protein kinase pathway versus the 2-5A pathway as mediators of the dsRNA-induced inhibition of translation in extracts of interferon treated cells.

2-5A trimer core monophosphate, p5'A2'p5'A2'p5'A, has been shown to prevent the inhibition of translation and degradation of RNA caused by 2-5A. Structure-activity relationships for this antagonistic action have shown that the 5'-monophosphate moiety is required for antagonistic activity. In contrast, the 2' terminal ribose is not required since it can be extensively modified without decrease in antagonistic capacity; however, complete removal of the 2' terminal adenosine leads to a significant, albeit not total, loss of antagonistic capacity, implying a function of the 2' terminal adenine moiety in endonuclease binding. The 2-4A-activated endoribonuclease will also tolerate the substitution of a 3',5-phosphodiester bond for at least one of the 2',5'-phosphodiester bonds without complete loss of antagonistic capacity. Chemical modification can potentiate the ability of oligonucleotides to reverse the action of 2-5A, and this increase in potency correlates with an increase in stability to degradation by enzyme activities present in cell-free extracts. Furthermore, the relative behavior of oligomers as 2-5A antagonists provides a guide to structures that will have increased protein synthesis inhibitory potency compared to 2-5A itself.

Up to 80-90% of the inhibition caused by poly(I)·poly(C) in extracts of interferon-treated L cells can be relieved with 2-5A derivatives such as 2-5A trimer core monophosphate, suggesting that the major mechanism mediating the translational inihibitory properties of poly(I)·poly(C) in extracts of interferon-treated cells is the 2-5A system.

The dsRNA analogue, poly(A)·poly(dUfl) [poly(2'-fluoro-2'-deoxyuridylic acid], is not an activator of 2-5A synthetase but does activate the protein kinase of both rabbit reticulocyte lysates and interferon-treated mouse L cell extracts to phosphorylate eIF-2α as well as the protein P_1. Analysis of 32P-phosphopeptides produced by proteolytic digests show that phosphosylation induced by poly(I)·poly(C) and poly(A)·poly(dUfl) in mouse cell extracts occurs on the same peptide(s) of eIF-2α. However, in contrast to the situation in rabbit reticulocyte lysates wherein both poly(I)·(C) and poly(A)·poly(dUfl) are potent inhibitors of protein synthesis, in extracts of interferon-treated L cells, only poly(I)·poly(C), but not poly(A)·poly(dUfl), causes inhibition of protein synthesis. These data show that eIF-2α and/or protein P1 phosphorylation does not correlate with inhibition of protein synthesis by poly(I)·poly(C) in extracts of interferon treated L cells.

Cordycepin analogs of 2-5A have been prepared by chemical means and have been prepared by chemical means and have been found to be unable to activate the 2-5A-dependent endoribonuclease of mouse L cells. Such analogs can, however, effectively bind to the endonuclease thus demonstrating that the 3'-OH groups of 2-5A are nto necessary for binding to the 2-5A-dependent endonuclease but are critical structural components in activation of the enzyme.

The Synthesis of Gephyrotoxin 223 Stereoisomers

The Lions-Willison indolizidine synthesis with butyraldehyde affords a 7:3 mixture of intermediate ketoamine diastereomers. On Clemmensen reduction, an olefin mixture results with one isomer (4) greatly predominating. The olefin mixture on catalytic reduction affords a 9:1 mixture of indolizidine diastereomers. The major isomer had nearly identical properties to these reported by Daly, *et al.* for the frog skin alkaloid 167B.

Central and Autonomic Nervous System Agents Based on the Piperidine Ring System

3-Acetyl-3,4-dehydropiperidines and related structures have been synthesized for study of the acetylcholine receptor. Arecolone and (-)-ferruginine methiodides are potent nicotinic agonists in the rectus abdominus muscle assay. 2-Artylbenzoates of piperidinyl and dialkylamino alcohols are active antiepileptics in the MES assay.

SECTION ON MICROANALYTICAL SERVICES AND INSTRUMENTATION

1. Service Functions and Instrumentation:

Basic research and service functions are performed by members of the Section. A major mission of the organization involves the instrumental and chemical analyses provided to scientists of the Laboratory of Chemistry, Laboratory of Bioorganic Chemistry, NIH, and to a limited extent to personnel of other government agencies. Approximately 30 of the more common elements and five functional groups are determined on a quantitative basis using ultramicro, micro, and semi-micro techniques as required. The materials analyzed include organic and inorganic research samples, commercial preparations and various biological specimens. Instrumental analyses include: GC/MS spectrometry, gas-liquid chromatography, GC with radioactive monitoring, infrared, nuclear magnetic resonance, amino acid analysis, ultraviolet and flame photometry. Assistance in the interpretation of spectra is rendered on request. (D. F. Johnson, H. J. C. Yeh, W. R. Landis, P. Parisius, B. Baer, A. Wong, N. Whittaker, B. Torain).

2. Applications of NMR in Biochemical and Biological Systems:

Phospholipase A_2 is an enzyme, present in high concentrations in snake and bee venoms and in mammalian pancreases, which catalyses the hydrolysis of the fatty acid ester bond at the 2-position of 3-sn-phosphoglycerides. The enzyme requires calcium ions for its catalytic activity and undergoes conformational changes upon binding of calcium ions. The nature of the active site and the mode of metal binding are of importance to the understanding of the nature of enzyme-substrate interactions. Various techniques, including NMR, sedimentation equilibrium, difference UV spectroscopy and equilibrium dialysis are being used to study this protein system. Several findings are obtained from these studies. 1) The active-site His-48 of these enzymes is buried in a hydrophobic region and is not accessible to the solvent water molecules. 2). The enzyme from N. naja atra venom exists predominately in a dimeric form which binds two calcium ions with different binding affinities (K_1 1.0×10^5 M^{-1} and K_2 5×10^2 M^{-1}). 3). Binding of calcium ions to the enzyme induces conformational perturbation at side chains of several residues, thus suggesting possible involvement of these residues in the metal-enzyme interaction. The residues involved in the interaction are one each of tyrosine, tryptophan and histidine; and probably more than one aspartic acid and leucine (or valine). 4). His-21, which is detected only when the pH is adjusted below 4, may be located near the region where the subunit interaction taking place. (H. J. C. Yeh and C. Chatterjee).

Carbon-13 NMR has been used to investigate the influence of the ring substituents on the directly bounded $^{15}N-^{13}C$ spin-spin coupling constants in a series of ring-substituted N,N-dimethylaniline-^{15}N derivatives and a series of nitrobenzene-^{15}N derivatives. In the nitrobenzenes, small changes in $^1J(^{15}N-^{13}C)$ are found which are attributed to the inductive effect of the substituents since steric inhibition of conjugation has little effect on the magnitude of the coupling. In contrast, steric inhibition of nitrogen lone-pair delocalization in N,N-dimethylaniline derivatives markedly reduces the value of $^1H(^{15}N-^{13}C)$. Theoretical calculations of $^1J(^{15}N-^{13}C)$ values for the two series of compounds were also made using standard INDO parameters and a "sum-over-states" perturbation approach. Fair agreement between the calculated and experimental values is found. (H.J.C. Yeh, T. Axenrod, C. M. Watnik, M. J. Wieder, S. Duangthai, G. A. Webb, S. Bulusu and M. M. King).

3. Nature of Steroid-Receptor Interactions:

Glucocorticoid steroid specific events are induced by nanomolar concentrations of hormone in responsive cells. Because the type of biological activity produced by a given steroid in a responsive cell (e.g., agonist or antagonist) is almost certainly determined by the individual steroid-receptor interactions, a molecular description of steroid-receptor interactions ultimately will be required for a better understanding of glucocorticoid hormone action. Unfortunately these studies have been hampered by the fact that receptor-steroid complexes dissociate (essentially immediately under denaturing conditions). In order to circumvent this obstacle, and to obtain chemial information about the steroid binding site of the receptor, we have been developing steroidal affinity labels. In our previous report we described our results in rat hepatoma tissue culture cells where dexamethasone 21-mesylate was found to be the first affinity label for glucocorticoid receptors. In order to strengthen this conclusion, and to determine the generality of dexamethasone 21-mesylate as an affinity label for glucocorticoid receptors, we have collaborated with Dr. Howard Eisen (NICHHD) for studies on the rat liver receptor.

Two convergent, independent lines of evidence were used to identify the rat liver glucocorticoid receptor. First, the electrophilic affinity label $[^3H]$dexamethasone 21-mesylate was used to label covalently the steroid specific binding site of the receptor. Second, antibodies to the rat liver receptor were used to isolate it from crude cytosols. A combination of these two techniques resulted in an immunochemical isolation of receptors covalently labeled with $[^3H]$dexamethasone 21-mesylate. (S. S. Stimons, Jr., H. J. Eisen and R. E. Schleenbaker).

Crude rat liver cytosol containing steroid-free receptors was chromatographed on Sephadex G-100 in buffer containing 20 mM sodium molybdate in order to remove small molecular weight components that react with dexamethasone 21-mesylate. Alternatively, the crude cytosol was treated with ammonium sulfate in the presence of 20mM sodium molybdate to precipitate the steroid-free receptor. Treatment of these steroid-free receptor solutions with $[^3H]$dexamethasone 21-mesylate, followed by analysis on denaturing polyacrylamide gels, reveals three major peaks of radioactivity at $M_r \simeq 90{,}000$, 67,000, and 52,000. The $M_r \simeq 67{,}000$ component was identified as albumin with the use of antibody to rat albumin. Competition experiments with dexamethasone demonstrated that only the $M_r \simeq 90{,}000$ moiety was saturated. The same $M_r \simeq 90{,}000$ moiety was adsorbed by immobilized antibody to the glucocorticoid receptor. Immobilized antibody could also be used to adsorb the steroid-free receptor from crude cytosols; treatment of the adsorbed receptor with $[^3H]$dexamethasone 21-mesylate resulted in specific labeling of an $M_r \simeq 90{,}000$ moiety. Thus, the steroid affinity labeling experiments and the immunochemical experiments concurrently identified the $M_r \simeq 90{,}000$ moiety as the glucocorticoid receptor. These studies firmly established the specificity of the antibody, and of the affinity-labeling steroid dexamethasone 21-mesylate for the rat liver glucocorticoid receptor. (S. S. Simons, Jr., H. J. Eisen and R. E. Schleenbaker).

Our previous application for a patent on the 21-mesylates of cortisol and dexamethasone was granted. New England Nuclear has been granted an exclusive licensing agreement for the production of $[^3H]$dexamethasone 21-mesylate. Thus there is now a ready, commerical supply of the radioactive steroidal affinity label for glucocorticoid receptor research. (S. S. Simons, Jr.)

We have also used mutant cells as probes of steroid-receptor interactions and steroid hormone action in whole cells. This study has been in collaboration with Dr. E. Brad Thompson (NCI). In particular, the glucocorticoid responses in two independently derived lines of rat hepatoma tissue culture cells (HTC and FU5-5) have been examined. FU5-5 cells exhibited induction of the enzyme tyrosine aminotransferse (TAT) at concentrations of dexamethasone that were ∿ 7-fold lower than that requred for HTC cells. FU5-5 cells also displayed substantial TAT induction with steroids that were partial agonists, or antagonists, in HTC cells. The increased sensitivity of FU5-5 cells was not, however, due to an increased affinity of FU5-5 cell receptors for dexamethasone, as determined from cell-free and whole cell binding experiments. The differential steroid sensitivity for TAT induction was observed with three other, structurally different glucocorticoids, thus apparently ruling out steroid metabolism in one of the cell lines as a cause. Also, induction of TAT in FU5-5 cells occurred at ∿ 9-fold lower steroid concentrations than were required for the induction of glutamine synthetase (GS) in the same cells. Thus the dose-response curves for TAT induction in HTC cells and for GS induction in FU5-5 cells are closely correlated with the saturation curve for whole cell steroid binding to receptor sites, while the dose-response curve for TAT induction in FU5-5 cells is shifted to lower steroid concentrations. This represents the first report of dissociation of two supposedly primary, glucocorticoid-induced functions and indicates that idential receptor-mediated mechanistic processess cannot be utilized by FU5-5 cells for the induction of TAT and GS. The involvement of second messengers or different nuclear processes are possible explanations for the unusual behavior of FU5-5 cells during glucocorticoid induction of TAT. (S. S. Simons, Jr., L. Mercier, and E. B. Thompson).

4. Characterization of the Dehydrogenase-Isomerase Enzymes and Metabolic Pathways in Tetrahymena Furgasoni (formerly Pyriformis).

The protozoan *Tetrahymena furgasoni* has steroid dehydrogenases that are similar in functional properties to the dehydrogenases of higher eukaryotes. Specifically, our prior identification of 4-androstene-3β,17β-diol and pregnenolone as transformation products from the incubation of *T. furgasoni* with testosterone and progresterone, respectively, indicated that this protozoan possesses a dehydrogenase that can reduce the C-3 carbonyl to a 3β-hydroxyl. The characterization of the dehydrogenase was pursued. The enzyme activity with both steroidal and non-steroidal substrates co-purified. Other evidence supporting the hypothesis that both activities reside in the same protein included: similar heat denaturation behavior; similar behavior with potential inhibitors; and the same pH optimum. Also a single protein band, obtained upon gel isoelectric focusing, was extracted and was found to contain dehydrogenase activity toward both steroidal and aliphatic secondary alcohols. The growth inhibition study confirmed that the transformation products pregnenolone and 4-androstene-3β,17β-diol were significantly less toxic than progesterone and testosterone, respectively, to cultures of *T. furgasoni* in log phase growth. Thus the dehydrogenase may function *in vivo* as a non-specific detoxication agent.

5. The Development of Methods and Materials for the Study of Medical Problems:

The object of this project is to make contributions to the investigation and solution of basic biological and medical problems by the application and development of chemical, physical and biological methods.

Lethality from cancer frequently results from metastases arising from cells of the primary tumor. The purposes of this study are: (a) to increase our understanding of the mechanisms operating at the various stages of metastasis, (b) to study the effects of chemicals and biologicals on metastases and their development, and (c) to apply the knowledge obtained thereby to the development of biological materials and chemical compounds of potential use in inhibiting the formation of metastases or selectively destroying them in human beings. The findings of these studies will also contribute to the investigation and increased understanding of other biological and medical problems.

During the metastatic process attachment of tumor cells to basement membrane must precede penetration; so differences in the abilities of tumor cells to attach to basement membrane may be a factor in differences in their metastatic potencies. Certain metastatic cells attach preferentially to type-IV collagen over other types of collagen or plastic. Two metastatic murine cell lines, B16-BL6 and T241-PMT, attach preferentially to type-IV collagen; and laminin, a high molecular weight glycoprotein found in basement membranes, increases the rate and extent of attachment. Cycloheximide inhibits the attachment of BL6 and PMT cells to type-IV collagen and the inhibition is overcome by added laminin. Antibodies to laminin also inhibit this attachment. Incubation of the cells with such antibodies prior to intravenous injection markedly reduces their metastatic potency; whereas preincubation of BL6 cells with laminin markedly increases their metastatic potency. These observations suggest that laminin is involved in attachment during the metastatic process.

Less than one percent of circulating tumor cells are successful in completing metastasis. The extravasation that is necessary to complete the process of hematogenous metastasis requires attachment and arrest of appropriate duration and character to permit survival of the tumor cells, breaching of the endothelium, and penetration of the basement membrane. The endothelium provides blood vessels with a nonthrombogenic surface, and if it is intact it is the first barrier to extravasation encountered by circulating tumor cells. Extravasation appears to be a very complex process and there is little understanding of the interactions of tumor cells with endothelial cells, subendothelium, blood cells, and soluble blood components invovled in the required arrest and invasion. The process will be understood more readily when the steps can be studies in vitro as separate phenomena. Versatile systems have been developed which should be useful in in vitro studies of this process and in other studies of endothelium and endothelial cells.

In the term placenta the amnion and chorion comprise the fetal membranes. The typical human amnion is less than 0.5 mm thick and it can be separated readily from the chorion. Morphologically it consists of a single layer of epithelial cells, a basement membrane, and a connective tissue stroma. Supported in a holder which allows collection of penetrating cells on a Millipore filter placed next to the stromal side of the membrane, the whole amnion can be used in migration, chemotaxis and invasion studies. By treatment with alkali (0.1% ammonium hydroxide) or detergent the amnion can be denuded of epithlial cells leaving basement membrane supported by the connective tissue stroma, which can be used in similar studies. Two kinds of endothelial cells, bovine capillary and human umbilical vein, have been applied to the denuded amnion supported in a holder and both gave grown to form confluent layers; presumably other endothelial cells will do likewise. Two human tumor cell lines, MCF-7 breast carcinoma and A-431 squamous carcinoma, in serum-free medium containing 0.1% fetuin have been applied to four-day monolayers of human endothelia cells growing on the amnion basement membrane-stroma. Both penetrated the system but at rates significantly reduced, after four days, relative to the rates of penetration of basement membrane-stroma alone. In studies thus far, only small proportions of human tumor cells applied to the amnion basement membrane or the membrane bearing a monolayer of bovine or human endothelial cells have penetrated the whole thickness of the barrier even after seven days. This may indicate that the systems are exercising a rigouous selection process which could make the results of the in vitro assays reliable measures of malignancy and metastatic potency. The appropriate system of membrane and/or cells supported by the holder and placed in a suitable chamber can be used in migration, chemotaxis and invasion stuides and in migration, chemotaxis and invasion studies and in studies of the interactions of cells, chemicals, or biologicals with epithelial, endothelial or other cells growing on basement membrane. Advantages of the method are the ease with which a large number of experiments can be done with one human amnion, the ease of quantitation and recoverability of viable penetrating cells, and the fact that systems homologous on heterologous in cells can be used. (Calvin M. Foltz and Lance A. Liotta (NCI)).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 19001-10 LC

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Immunochemistry and Reaction of Carbohydrates

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. P. J. Glaudemans	Chief, Section on Carbohydrates	NIADDK/LC
Other:	A. K. Bhattacharjee	Visiting Scientist	NIADDK/LC
	G. Ekborg	Visiting Associate	NIADDK/LC
	B. Vranesic	Visiting Fellow	NIADDK/LC
	E. Zissis	Chemist	NIADDK/LC

COOPERATING UNITS (if any)

Dr. M. Potter (NCI); Dr. J. E. Bennett and K. J. Kwon-Chung (NIAID;
Ms. E. Mushinsky (NCI)

LAB/BRANCH

Laboratory of Chemistry

SECTION

Section on Carbohydrates

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.5	4.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES XX (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The section works on a) the interaction of biologically important polysaccharides with antibodies; b) the structure and nature of antibodies; c) the preparation of monoclonal immunoglobulins to specific determinants.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AM 19200-32LC

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Biological Testing for Antinociceptive Activity, Acute Toxicity and Dependence Liability

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. E. Jacobson	Research Chemist	NIADDK-LC
OTHER:	E. L. Atwell	Medical Biology Technician	NIADDK-LC

COOPERATING UNITS (if any)

Deparment of Pharmacology of the University of Michigan and the Medical College of Virginia, and the Committee on Problems of Drug Dependence, Inc.

LAB/BRANCH

Laboratory of Chemistry

SECTION

Medicinal Chemistry

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	0.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Animal tests are used to determine the antinociceptive activity of potential analgesic agents. Acute toxicities have, also, been determined on a number of compounds with disparate biological activities. About 40 compounds have been sent to out cooperating units for further investigation; others have been sent to NCI for screening.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AM 19202-09LC

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)
Synthesis and Evaluation of Potential CNS, Antiinflammatory and Anticancer Agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	K. C. Rice	Research Chemist	NIADDK-LC
OTHER:	M. F. Rafferty	Guest Worker	NIADDK-LC
	A. E. Jacobson	Research Scientist	NIADDK-LC

COOPERATING UNITS (if any)
University of Colorado (B. Hoffer); NIADDK-LBC (P. Skolnick) NIMH-CP (S. Paul), University of Michigan (J. Woods).

LAB/BRANCH
Laboratory of Chemistry

SECTION
Medicinal Chemistry

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The recently synthesized enantiomers of 1-(1-phenylcyclohexyl)-3-methyl-piperidine (PCMP) were used to detect stereospecificity of displacement of high affinity [^{3}H]-PCP in rat olefactory bulk slices. Long acting and essentially irreversible binding of 2-nitro and 2,8-dinitroimipramine to high affinity binding sites on human platelets was observed suggesting that these compounds will be of value in isolation and purification of these binding sites. Antagonism of enkephalin-induced changes in spontaneous cell firing in vivo by (+)-and(-)-naloxone was studied.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AM 19212-06LC

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Practical Total Synthesis of Codeine and Other Important Opium Alkaloids

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. Brossi	Chief, Medicinal Chemistry	NIADDK-LC
OTHER:	F. Hsu	Staff Fellow	NIADDK-LC
	K. C. Rice	Research Chemist	NIADDK-LC

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Chemistry

SECTION
Medicinal Chemistry

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0	0	

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER
☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is presently inactive.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 19216-06LC

PERIOD COVERED

October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Further Structural Variations in Colchicine

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Arnold Brossi	Visiting Scientist	NIADDK-LC
OTHERS:	Peter Buchs	Research Fellow	NIADDK-LC
	Padam Sharma	Research Fellow	NIADDK-LC
	Arthur E. Jacobson	Senior Scientist	NIADDK-LC
	Louise Atwell	Technician	NIADDK-LC

COOPERATING UNITS (if any)

Maria A. Iorio, Laboratory of Pharmaceutical Chemistry, Istituto Superiore di Sanita, 00161 Rome, Italy. Colin F. Chignell, Laboratory of Env. Biophys., NIEHS, Research Triangle Park, North Carolina 27709

LAB/BRANCH

Laboratory of Chemistry

SECTION

Medicinal Chemistry

INSTITUTE AND LOCATION

NIADDK, NIH, Betehsda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Colchicide, originally prepared by Rapoport, binds well to tubulin and is active in the leukemia P388 assay in vivo. It is thus demonstrated that the 10-OMe group in colchicine is not essential for tubulin binding and antimitotic activity. The substitution on the aromatic ring and the nature of the C-7 side chain on the other hand are critical. Several novel N-acyl- and N-aroyldeacetylcolchicines were prepared, and some were found to be highly active antimitotic agents. The N-sinapinoyldeacetylcolchicine showed good uv-absorption and will be tested as a potential candidate to mark the colchicine binding site of tubulin. The position of the double bond in dehydrodeacetamidocolchicine prepared from colchicine could be assigned to the C-5:C-6 position. Deacetamidocolchicine, although highly potent in vitro, does not seem to be very active in vivo.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 19219-05LC

PERIOD COVERED

October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Synthesis of 3-Deoxydihydromorphine and Related Compounds

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Arnold Brossi	Visiting Scientist	NIADDK-LC
OTHER:	Kenner C. Rice	Research Chemist	NIADDK-LC
	Fu Lian Hsu	Senior Staff Fellow	NIADDK-LC
	Helmut Schmidhammer	Visiting Scientist	NIADDK-LC

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Chemistry

SECTION

Medicinal Chemistry

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.75	1.75	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Total synthesis of (±)-4-hydroxy-N-methylmorphinan-6-one and its O-methyl ether has been accomplished. The structure of the by-products obtained during the O-alkylation of (+)-2,4-dihydroxy-N-formylmorphinan-6-one with 1-chloro-5-phenyl-1H-tetrazole was established. An improved synthesis of this series of ketomorphinans is now at hand by eliminating the 2-OH group at the stage of the secondary alcohol, rather than the ketone.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 19222-05 LC

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Retinylidene 1,3-Diketones as Potential Antitumor Agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. Brossi	Chief, Medicinal Chemistry	NIADDK-LC
OTHER:	N. Acton	Guest Worker	NIADDK-LC

COOPERATING UNITS (if any)

M. Sporn, NIH Cancer Institute

LAB/BRANCH

Laboratory of Chemistry

SECTION

Medicinal Chemistry

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0	0	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project has been terminated.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 19223-05LC

PERIOD COVERED

October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Tetrahydropapaveroline ("THP")

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. Brossi	Visiting Scientist	NIADDK-LC
OTHER:	K. C. Rice	Research Chemist	NIADDK-LC
	P. Buchs	Visiting Fellow	NIADDK-LC
	F. L. Hsu	Senior Staff Fellow	NIADDK-LC
	H. Schmidhammer	Visiting Scientist	NIADDK-LC
	P. Sharma	Visiting Fellow	NIADDK-LC

COOPERATING UNITS (if any)

P. Skolnick, M. Schweri and J. Daly, Laboratory of Organic-Biochemistry, NIADDK
J. V. Silverton, R. Potenzone, Jr., Heart, Lung and Blood Inst., NIH

LAB/BRANCH

Laboratory of Chemistry

SECTION

Medicinal Chemistry

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.5	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Synthesis of (+)-6'-methylnorreticuline and its R-(-)-enantiomer has been accomplished from the berbine alkaloids (+)-coreximine and its R- (+)-enantiomer. The 6'-methyl-THP analogs were obtained by O-demethylation. This synthesis also provided an entry into rare tetracyclic isoquinoline alkaloids of the benzobyclo-hepteno group. In another approach optically active 6'-bromo analogs of THP were prepared and found to be more selective in interacting with dopaminergic and adrenergic receptor systems than their desbromo analogs. An optically active rotamer of 6'-bromo-N-formylnorreticuline was subjected to single crystal X-ray analysis.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AM 19224-04LC

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Microbiological Oxidation of 6-Ketomorphinans and 3-Deoxymorphines

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. Brossi	Chief, Medicinal Chemistry	NIADDK-LC
OTHER:	H. Schmidhammer	Visiting Scientist	NIADDK-LC

COOPERATING UNITS (if any)
Prof. K. Kieslich, GBF, Gesellschaft für Biotechnologische Forschung, Braunschweig, West Germany

LAB/BRANCH
Laboratory of Chemistry

SECTION
Medicinal Chemistry

INSTITUTE AND LOCATION
NIADDK-LC, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0	PROFESSIONAL: 0	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER
☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project has been terminated.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AM 19226-04LC

PERIOD COVERED

October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Synthesis and Pharmacology of Irreversible Inhibitors of the Benzodiazepine Receptor.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	K. C. Rice	Research Chemist	NIADDK-LC

COOPERATING UNITS (if any)

NIMH-BP (J. Tallman), NIMH-CP (S. Paul, J. Crawley), NIADDK-LBC (P. Skolnick) NINCDS-LN (J. Barker), U. of Alabama (S. Barker), U. of Wisconsin (J. Cook).

LAB/BRANCH

Laboratory of Chemistry

SECTION

Medicinal Chemistry

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Synthesis and brain in vitro binding studies of a broad series of β-carbolines and related compounds have established a relatively clear structural-activity relationship in this series. Optimum binding was observed in the fully aromatic β-carboline series when an ester or ketone carbonyl group was present at C-3. The 3-acetyl and 3-formyl derivatives were benzodiazepine antagonists in vivo (mice). A biomimetic Pictet-Spengler approach was used to prepare several imidazopyridines which showed moderate affinity for the benzodiazepine receptor.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 19228-04LC

PERIOD COVERED

October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Aromatic Oxygenation and Antinociception in 6-Ketomorphinans

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Arnold Brossi	Visiting Scientist	NIADDK-LC
OTHER:	Arthur E. Jacobson	Senior Research Scientist	NIADDK-LC
	Louise Atwell	Technician	NIADDK-LC
	Helmut Schmidhammer	Visiting Scientist	NIADDK-LC
	Fu-Lian Hsu	Senior Staff Fellow	NIADDK-LC
	Maria D. Rozwadowska	Visiting Scientist	NIADDK-LC

COOPERATING UNITS (if any)

F. Medzihradsky, Dept. of Biol. Chem., University of Michigan, Medical School; Judith Flippen-Anderson, Lab. of the Structure of Matter, Naval Research Lab., Washington, D. C.

LAB/BRANCH
Laboratory of Chemistry

SECTION
Medicinal Chemistry

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 3.4	PROFESSIONAL: 3.0	OTHER: 0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Total synthesis of (-)-2-hydroxy-N-methylmorphinan-6-one and its (+)-enantiomer has been achieved. Deoxygenation of these isomers afforded the optically active, aromatic unsubstituted 6-ketomorphinans. A chemical converson of a (-)-4-hydroxy-substituted 6-ketomorphinan, prepared from nat. morphine, into its (-)-1-hydroxy isomer and its O-methyl ether has been accomplished. The 1- and 2-oxygenated ketomorphinans showed remarkably little antinociception, whereas the aromatic unsubstituted (-)-isomer was highly active. It is now established that the oxygen substitution in 6-ketomorphinan is best at C-3, C-4 and C-3,4. The O-methyl ethers of these phenolic ketomorphinans are more potent than the parent phenols in the 6-ketomorphinan series, and less potent in the morphinan series. N-Phenethylation and N-allylation or N-cycloalkylation increased potency or antagonism response.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AM 19233-03LC

PERIOD COVERED

October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Total Synthesis of Opioids via Dihydrothebainone and Derivatives

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: K. C. Rice	Research Chemist	NIADDK-LC
OTHER: None		

COOPERATING UNITS (if any)

Research Triangle Institute, Research Triangle Park, N. C. (F.I. Carroll)

LAB/BRANCH

Laboratory of Chemistry

SECTION

Medicinal Chemistry

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Md. 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Additional refinements in the synthetic route (previsously reported) have been demonstrated which include a simple racemization of the undesired enantiomer of an intermediate and vastly improved conditions for the Birch reduction. Introduction of an N-chloroacetyl protecting function has provided a highly crystalline easily isolated, Grewe cyclization product which can be readily be cleaved by acid hydrolysis nearly quantitatively to chiral 1-bromonordihydrothebainone.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 19234-02LC

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Novel Approaches Towards the Synthesis of Pyrrolizidine Alkaloids and Anatoxin-a

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. Brossi	Chief, Medicinal Chemistry	NIADDK-LC
Other:	P. Buchs	Visiting Fellow	NIADDK-LC

COOPERATING UNITS (if any)

Drs. Flippen-Anderson and Isabella Karle, Naval Research Laboratory, Washington, D. C.

LAB/BRANCH

Laboratory of Chemistry

SECTION

Medicinal Chemistry

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0	PROFESSIONAL: 0	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project has been incorporated with Z01 AM 19243-01

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AM 19235 -01LC

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Characterization of Opiate Receptors Using Nonrigid Irreversible Inhibitors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	K. C. Rice	Research Chemist	NIADDK-LC
OTHER:	A. E. Jacobson	Research Chemist	NIADDK-LC
	T. R. Burke, Jr.	Staff Fellow	NIADDK-LC

COOPERATING UNITS (if any)

W. Klee, NIMH, NIH, Bethesda, Md.

LAB/BRANCH
Laboatory of Chemistry

SECTION
Medicinal Chemistry

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ XX (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In order to gain insight into the function and role of opiate receptor subpopulations in the mediation of analgesia, addiction, euphoria and tolorence, we have prepared specific, irreversible inhibitors of these receptors based on the patent, nonrigid analgesics fentanyl and etonitazene. In these studies, we have obtained at least one compound which preferentially inhibits mu receptors and one which preferentially inhibits delta receptors.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AM 19236-01LC

PERIOD COVERED

October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Characterization of Opiate Receptors Using Rigid Probes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. E. Jacobson	Research Chemist	NIADDK-LC
OTHER:	B. S. Bajwa	Guest Worker	NIADDK-LC
	K. C. Rice	Research Chemist	NIADDK-LC
	W. A. Klee	Research Biochemist	LGCB-NIMH-ADAMHA

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Chemistry

SECTION

Medicinal Chemistry

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES x☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A series of relatively rigid molecules, from the endoethenoripavine, endoethanooripavine, and benzomorphan families of opioids known to bind to opiate receptors from rat brain cerebrum homogenates and neuroblastoma-glioma hybrid cells, were converted to ligands potentially capable of irreversible interaction with those receptors. At least one of them has been found to act as an irreversible binding ligand for the mu opiate receptor and is being used to differentiate the mu receptors, known to mediate analgesia, from other opiate receptors.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 19237-01LC

PERIOD COVERED

October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Topological Characterization of Opiate Receptors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	K. C. Rice	Research Chemist	NIADDK-LC
OTHER:	T. R. Burke, Jr.	Staff Fellow	NIADDK-LC
	A. E. Jacobson	Research Chemist	NIADDK-LC

COOPERATING UNITS (if any)

W. Klee, NIMH, Bethesda, Md.

LAB/BRANCH

Laboratory of Chemistry

SECTION

Medicinal Chemistry

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Md. 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES xx ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Conformationally restricted congeners of opiate agonists are in preparation and will be utilized in a number of _in vivo_ and _in vitro_ systems to map the topological requirements for opiate activity.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 19238-01LC

PERIOD COVERED

October 1, 1981 through Septermber 30, 1982

TITLE OF PROJECT (80 characters or less)

Synthesis of 5,6,7,8-Tetramethoxy-substituted Cactus Alkaloids

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Arnold Brossi	Visiting Scientist	NIADDK-LC
OTHER:	Kimio Takahashi	Research Fellow	NIADDK-LC

COOPERATING UNITS (if any)

Judith L. Flippen-Anderson and I. Karle, Laboratory of the Structure of Matter, Navy Research Laboratories, Washington, D. C.

LAB/BRANCH

Laboratory of Chemistry, NIADDK

SECTION

Medicinal Chemistry

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The Cactus alkaloids weberine, norweberine and their 1-methyl congeners are 5,6,7,8-tetramethoxy-substituted 1,2,3,4-tetrahydroisoquinolines. They were synthesized from 2,3,4,5-tetramethoxybenzaldehyde and the derived phenethylamine by Pictet-Spengler and Bischler-Napieralski synthesis. Totally synthetic weberine was identical with the natural alkaloid isolated from the Mexican cactus Pachycereus weberi. Single crystal X-ray analysis of representative TIQ revealed that all four methyl groups attached to the aromatic oxygens are out-of the plane of the aromatic ring, a novel and intersting finding.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 19239-01LC

PERIOD COVERED

October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Characterization of the Antitrypanosomal Factor from Pseudomonas fluorescens

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	K. C. Rice	Research Chemist	NIADDK-LC
OTHER:	M. F. Rafferty	Guest Worker	NIADDK-LC

COOPERATING UNITS (if any)

LPD, NIAID

LAB/BRANCH

Laboratory of Chemistry

SECTION

Medicinal Chemistry

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Md. 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES xx☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Pseudomonas fluorescens was recently observed to secrete a substance which is toxic for Trypanosoma cruzi, the causative organism for South American sleeping sickness (Chagas' Disease). We are currently pursuing the isolation of this toxin via gel filtration and HPLC.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AM 19240-01LC

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Synthesis and Biological Evaluation of Tetramethoprim and Pentamethoprim

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Arnold Brossi	Visiting Scientist	NIADDK-LC
OTHER:	Kimio Takahashi	Research Fellow	NIADDK-LC

COOPERATING UNITS (if any)

Dr. Raether, Hoechst Pharmaceuticals, Frankfurt, Germany. Judith L. Flippen-Anderson, Laboratory of the Structure of Matter, Navy Laboatories, Washington, D. C.

LAB/BRANCH
Laboratory of Chemistry, NIADDK

SECTION
Medicinal Chemistry

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Diaveridine (3,4-OMe) and trimethoprim (3,4,5-OMe) are two highly potent systemic antibacterials, particularly in combination with sulfa drugs. We have now prepared tetramethoprim (2,3,4,5-OMe) and pentamethoprim (2,3,4,5,6-OMe) from the corresponding benzaldehydes by conventional synthesis. Tetramethoprim, when compared with trimethoprim, was much less active in vitro against Str. pyog., E. Coli and Salm. typhimurium. The importance of the aromatic substitution is thus demonstrated and the structures of the novel compounds will be analyzed by single crystal X-ray analysis.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 19241-01LC

PERIOD COVERED

October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Relationship of Benzomorphan Analgesics to Dissociative Anesthetics

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. E. Jacobson	Research Chemist	NIADDK-LC
OTHER:	B. S. Bajwa	Guest Worker	NIADDK-LC
	K. C. Rice	Research Chemist	NIADDK-LC
	M. Rafferty	Guest Worker	NIADDK-LC
	J. V. Silverton	Research Chemist	NHLBI-LC

COOPERATING UNITS (if any)

Department of Pharmacology, University of Michigan Medical School

LAB/BRANCH

Laboratory of Chemistry

SECTION

Medicinal Chemistry

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.75	0.75	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

X-ray crystallographic data on dexoxadrol, a dissociative anesthetic known to bind to the phencyclidine receptor obtained from rat and pigeon brain membranes, indicated that it could overlap two significant areas of benzomorphan analgesics when observed three-dimensionally using computer graphics. These data are being used to synthesize "analgesics" which would overlap three-dimensionally with three points of possible significance in dexoxadrol, in order to gain information about the PCP receptor.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AM 19242-01LC

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Chemistry of Antimalarial Dihydro-s-triazines

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Arnold Brossi	Visiting Scientist	NIADDK-LC
OTHER:	Nancy Acton	Guest Worker	NCI
	Balbir Bajwa	Guest Worker	NIADDK

COOPERATING UNITS (if any)

C. Canfield, Walter Reed Army Institute of Research, Div. of Exp. Therapeutics.

LAB/BRANCH
Laboratory of Chemistry

SECTION
Medicinal Chemistry

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The oxydihydrotriazine antimalarial WR-99210 is active against strains of Plasmodium berghei resistent to cycloguanil and pyrimethamine. The presence of a masked hydroxylamine function and a 2,4,5-trichlorophenoxy residue in this interesting compound are unappealing and suggested a chemical investigation. Treatment of WR-99210 with refluxing 1N HCl fragments the molecule into an acetone oxime ether derivative and a C_2N_4-moiety not yet identified. Further hydrolysis of the acetone oxime ether unmasks the hydroxylamine unit and afforded the corresponding hydroxylamine ether derivative. Cyclization of the former with cyano-guanidine gave the biguanide and its cyclization with acetone gave chemically pure WR-99210. The antimalarial activity of chemically pure WR-99210 and its biguanide precursor will be measured.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 19243-01 LC

PERIOD COVERED

October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Naturally Occuring Toxins as Probes for Receptors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. Brossi	Chief, Med. Chemistry	NIADDK-LC
OTHER:	K. Takahashi	Visiting Fellow	NIADDK-LC
	A. E. Jacobson	Research Chemist	NIADDK-LC
	P. Buch	Visiting Fellow	NIADDK-LC

COOPERATING UNITS (if any)

J. L. Flippen-Anderson, Laboratory of the Structure of Matter, Navy Research Laboratories, Washington, D. C.; J. V. Silverton, National Heart, Lung and Blood Institute

LAB/BRANCH

Laboratory of Chemistry

SECTION

Medicinal CHemistry

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐XX (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Regioselective synthesis of (Z)-5-alkyl-pyrrolizidin-3-ones and their (E)-isomers has been accomplished. This material will be used for a total synthesis of ant toxins. Some precursors serve as intermediates for a total synthesis of anatoxin-a. Repetition of the Edwards synthesis starting from natural cocaine has given material needed for testing and for derivatization.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AM 19401-17LC

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Natural Products as Agonists, Antagonists, Desensitizers and Probes for Receptors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Bernhard Witkop	Chief, LC	NIADDK-LC

COOPERATING UNITS (if any) O. Yonemitsu; Y. Kanaoka & T. Iwakuma, Univ. of Hokkaido, Sapporo, Japan; Masako Nakagawa, Faculty of Pharmaceutical Sciences, Chiba Univ. Japan; I. L. Karle, U.S. Naval Research Lab., Wash. D.C., Prof. E. X. Albuquerque, & Dr. C. Spivak, Univ. of Md. Med. School, E. Gössinger, Univ. of Vienna, Robert Aronstam, Univer. Ga.; Dr. Tamara Gund, New Jersey Inst. Tech., Newark, N.J.

LAB/BRANCH
Chemistry

SECTION
Metabolites

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.5	.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The Chief Investigator - largely with extramural support - has kept up a widely diversified program, international and interdisciplinary in character, involving binding studies of agonists, electrophysiology of ion flux, photochemistry of drug models, history of science and international scientific exchange.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 19402-09 LC

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Interferon Induction and Action. The Antiviral Activity of Nucleoside Analogs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Paul F. Torrence	Research Chemist	NIADDK/LC
Other:	Margaret I. Johnston	Senior Staff Fellow	NIADDK/LC
	Jiro Imai	Visiting Fellow	NIADDK/LC
	Krystyna Lesiak	Visiting Fellow	NIADDK/LC

COOPERATING UNITS (if any) R.M. Friedman, USUHS; H. Jacobsen, LEP/NIADDK; B. Safer, LMH/NHLBI; E. DeClercq, Univ. Leuven, Belgium; P. Lengyel, Yale University, New Haven, Conn.; H. Sawai, University of Tokyo

LAB/BRANCH
Laboratory of Chemistry

SECTION
Section on Metabolites

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.0	4.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Interferon-induced enzyme activities such as the oligo(2'→5')adenylate synthetase, the 67K dalton protein kinase and oligo(2'→5') A phosphodiesterase are investigated with a goal of understanding their role in the action of interferon, the induction of interferon by double-stranded RNA and, perhaps, control of cell growth and differentiation.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AM 19408-09-LC

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Central and Autonomic Nervous System Agents Based on the Piperidine Ring System

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	James A. Waters	Research Chemist	NIADDK/LC
Other:	C. E. Spivak	Pharmacologist	Univ. Md. Baltimore
	B. Witkop	Chief, LC	NIADDK/LC

COOPERATING UNITS (if any) Antiepileptic Drug Development Program, Epilepsy Branch, NINCDS, NIH

LAB/BRANCH

Laboratory of Chemistry

SECTION

Section on Metabolites

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 1.0	PROFESSIONAL: 1.0	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

3-Acetyl-3,4-dehydropiperidines and related structures have been synthesized for study of the acetylcholine receptor. Arecolone and (-)-ferruginine methiodides are potent nicotinic agonists in the rectus abdominus muscle assay. 2-Arylbenzoates of piperidinyl and dialkylamino alcohols are active antiepileptics in the MES assay.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AM 19410-04 LC

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

The Synthesis of Gephyrotoxin 223 Stereoisomers

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI;	Thomas F. Spande	Research Chemist	NIADDK-LC

COOPERATING UNITS (if any)

John W. Daly, Chief, Laboratory of Bioorganic Chemistry, NIADDK

LAB/BRANCH
Laboratory of Chemistry

SECTION
Section on Metabolites

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	1	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The Lions-Willison indolizidine synthesis with butyraldehyde affords a 7:3 mixture of intermediate ketoamine diastereomers. On Clemmensen reduction, an olefin mixture results with one isomer (4) greatly predominating. The olefin mixture on catalytic reduction affords a 9:1 mixture of indolizidine diastereomers. The major isomer had nearly identical properties to these reported by Daly, et al. for the frog skin alkaloid 167B.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 19603-06 LC

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Fluoro Analogs of Enzyme Substrates

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Louis A. Cohen	Chief, Biochemical Mechanisms	NIADDK-LC
OTHER:	Kenneth L. Kirk	Research Chemist	NIADDK-LC
	Kazuyuki Takahashi	Visiting Fellow (Arr. 8/80)	NIADDK-LC
	Virender Labroo	Visiting Fellow (Arr. 4/81)	NIADDK-LC

COOPERATING UNITS (if any) P. Dannies, Dept. of Pharmacology, Yale Medical School, New Haven, Conn.

LAB/BRANCH

Laboratory of Chemistry

SECTION

Biochemical Mechanisms

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.1	1.9	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Ring-fluorinated analogs of various metabolically significant imidazoles have been found to have interesting and useful activities as specific enzyme inhibitors and as antibacterial, antiviral and antileukemic agents. Efforts to expand the variety of analogues to include 2,4-difluoroimidazoles and the isomeric fluoroaminoimidazoles had been thwarted because of the relative instabilities of these polyfunctional systems. Members of these series containing carboxylic esters or carboxamide at C-5 have now been prepared for biological evaluation. Synthesis of the corresponding ribosides is being explored. Both 2-fluoro-his-TRF and 4-fluoro-his-TRF have been synthesized from the corresponding amino acids. These analogs of the peptide hormones are being evaluated for receptor binding and prolactin release capabilities.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AM 19604-12 LC

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

General Principles of Enzyme Catalysis and Simulation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Louis A. Cohen	Chief, Section on Biochem. Mech.	NIADDK-LC
OTHER:	Michael King	Guest Worker	GWU
	Wieslaw Antkowiak	Visiting Scientist	NIADDK-LC

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Chemistry

SECTION
Section on Biochemical Mechanisms

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.6	1.3	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ XX(c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We have advanced the theory that a major portion of enzymatic catalysis is achieved by activation of the substrate through binding. To support the theory, we have synthesized a large variety of test-tube models which simulate the bound substrate by being frozen in a single, favorable conformation and by having the interacting functions brought into the closest possible juxtaposition (stereopopulation control). These compounds undergo intramolecular reactions at rates comparable to those catalyzed by enzymes, sometimes even too fast to measure. To prove that our model compounds are truly frozen in unique conformations in solution, we have initiated efforts to demonstrate that these molecules possess molecular chirality, and that the resolved enantiomers do not racemize at elevated temperatures. Furthermore, log k for enhanced cyclization is a function of the Van der Waals size of restricting groups and is independent of electronic effects.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 19605-06 LC

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Chemistry of Substituted Imidazoles

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Louis A. Cohen	Chief, Biochemical Mechanisms	NIADDK-LC
OTHER:	Kenneth L. Kirk	Research Chemist	NIADDK-LC
	Robert Jerussi	Guest Worker (FDA)	NIADDK-LC
	Stuart Cohen	Guest Worker (EPA)	NIADDK-LC
	Virender Labroo	Visiting Fellow (Arr. 4/81)	NIADDK-LC
	Ron Breiman	Visiting Fellow (Arr. 8/81)	NIADDK-LC

COOPERATING UNITS (if any) Hiroshi Kimoto, Industrial Research Institute, Nagoya, Japan; Robert Henkin, Georgetown Univ. Hospital, Washington, D. C.

LAB/BRANCH

Laboratory of Chemistry

SECTION

Section on Biochemical Mechanisms

INSTITUTE AND LOCATION NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	1.0	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Our demonstrations of the widespread and valuable properties of fluoroimidazoles as biological substrates and inhibitors have provided the impetus for the synthesis and evaluation of other substituted imidazoles in biological systems. A host of previously unknown analogues of histamine and histidine have now been obtained with substituents at C-2, C-4, or both. Novel synthetic methods have been developed to provide analogues containing halogen, amino, formyl, cyano, nitro, carboalkoxy, and trifluoromethyl groups. The last compounds were found to be particularly labile above pH 8 and are being investigated as receptor affinity labels for use in vivo. Toward this end, the isomeric trifluoromethyl-His analogs of TRF have been synthesized and are being evaluated for prolactin release and for irreversible binding to TRF receptors. A linear free energy correlation has been achieved (by computer analysis) for predicting the electronic effects of other substituents on the reactivity of the trifluoromethyl group in various imidazoles. A unique method has been developed for the synthesis of 2-hydroxylaminoimidazoles, compounds which are expected to be selectively cytotoxic for cancer cells.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 19606-06 LC

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Halogenated Biogenic Amines in Biochemistry and Pharmacology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Kenneth L. Kirk	Research Chemist	NIADDK-LC
Other:	Rosa Antkowiak	Guest Worker	NIADDK-LC
	George Chen	Guest Worker	NIADDK-LC
	Konstantin Buchhold	Biological Aide	NIADDK-LC

COOPERATING UNITS (if any) C. R. Creveling, LBC, NIADDK; D. Thakker, LBC, NIADDK; U. Weiss, LCP, NIADDK; J. Costa, C. C. Chiueh, NIMH; E. Sokoloski, NHLBI; B. Hoffer, T. V. Dunwiddie, U. of Colorado; D. Diffley, Johns Hopkins; M. Dratman J. Gordon, V. A. Hospital Philadelphia, PA; H. Kimoto, Nagoya, Japan.

LAB/BRANCH

Laboratory of Chemistry

SECTION

Section on Biochemical Mechanisms

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Md. 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.6	0.8	0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Fluorinated analogs of norepinephrine, isoproterenol, phenylephrine, tyramine, dopamine, melatonin, serotonin, DOPA, α-methyl DOPA, reserpine, normetanephrine and norparanephrine have been synthesized. Biochemical and biophysical studies on these analogs have provided important information concerning structural requirements for norepinephrine in interaction with α- and β- adrenergic receptors, and the role of phenolic pK and lipophilicity in determining activity of biogenic amines.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AM 19801-37LC

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Service Functions and Instrumentation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. F. Johnson	Chief, Micro. Ser. & Instr.	NIADDK-LC
Other:	H. J. C. Yeh	Research Chemist	NIADDK-LC
	W. R. Landis	Chemist	NIADDK-LC
	P. Parisius	Chemist	NIADDK-LC
	B. Baer	Chemist	NIADDK-LC
	A. Wong	Chemist	NIADDK-LC
	N. Whittaker	Chemist	NIADDK-LC
	B. Torain	Biol. Lab. Tech.	NIADDK-LC

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Chemistry

SECTION
Micronanalytical Services and Instrumentation

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
7.0	7.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES XX☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Basic research and service functions are performed by members of the Section. A major mission of the organization involves the instrumental and chemical analyses provided to scientists of the Laboratory of Chemistry, Laboratory of Bioorganic Chemistry, NIH, and to a limited extent to personnel of other government agencies. Aproximately 30 of the more common elements and five functional groups are determined on a quantitative basis, using ultramicro, micro, and semimicro techniques as required. The materials analyzed include organic and inorganic research samples, commercial preparations and various biological specimens. Instumental analyses include: GC/MS spectrometry, gas-liquid chromatography, GC with radioactive monitoring, infrared, nuclear magnetic resonance, amino acid analysis, ultraviolet and flame photometry. Assistance in the interpretation of spectra is rendered on request.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AM 19802-09 LC

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Characterization of an Alcohol Dehydrogenase from Tetrahymena Furgasoni (formerly Pyriformis)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Nancy Lamontagne	Research Chemist	NIADDK-LC
Other:	David F. Johnson	Section Chief	NIADDK-LC

COOPERATING UNITS (if any)
Chester E. Holmlund, Dept. of Chem., University of Maryland, College Park, Md.

LAB/BRANCH
Laboratory of Chemistry

SECTION
Microanalytical Services and Instrumentation

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0.1	PROFESSIONAL: 0.1	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Our objective was to isolate a dehydrogenase from a cell-free extract of T. Furgasoni. The partially purified enzyme demonstrated a substrate specificity for secondary alcohols, therefore the kinetics with both non-steroidal alcohols were studied. Characterization of the secondary alcohol dehydrogenase included pH optimum, molecular weight, effect of inhibitors, and heat. A growth-inhibition study was also done to investigate whether the in vivo reduction of the ketone at the C-3 of a steroid was a detoxication mechanism.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AM 19803-09LC

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Applications of NMR in Biochemical and Biological Systems

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H.J.C. Yeh	Research Chemist	NIADDK-LC
Other:	C.L. Chatterjee	Visiting Fellow	NIADDK-LC

COOPERATING UNITS (if any) C.C. Yang (Natl. Tsing-Hua University, Taiwan); T. Axenrod, C.M. Watnick and M.J. Wieder (City College of City Univ. of New York); S. Duangthai and G.A. Webb (Univ. of Surrey, England); S. Bulausu (U.S. Army Armament Research and Development Command); M.M. King (George Washington Univ., Washington, D.C.)

LAB/BRANCH
Laboratory of Chemistry

SECTION
Microanalytical Services and Instrumentation

INSTITUTE AND LOCATION
NIADDK,NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

As part of the project to develop and the NMR method in biochemical and biological systems we have used this techniques together with ultracentrifugation, difference UV spectroscopy and equilibrium dialysis methods to study the structure and conformation of phospholipase A2 of snake vemons and to investigate their properties such as monomer-dimer transtitions and enzyme-metal interactions. The current data show that the phospholipase A2 and N. naja atra venom exits predominantly in a dimeric form which binds two calcium ions with different binding affinities. Results of these studies reveal the hydrophobic nature of the active center of the enzyme and yield information about the amino acid residues which are near or the calcium binding site.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 19804-09LC

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

The Development of methods and materials for the study of Medical Problems.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Calvin M. Foltz	Research Chemist	NIADDK/LC
Other:	Lance A. Liotta	Pathologist	NCI-LPP

COOPERATING UNITS (if any)

National Cancer Institute

LAB/BRANCH

Laboratory of Chemistry

SECTION

Microanalytical Services and Instrumentation

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS XX (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The primary goal of this work is to contribute to the investigation and solution of basic biological and medical problems by the application and development of chemical, physical and biological methods. This is being done by the application of chemistry to the isolation and study of biomolecules, by the synthesis and study of chemical compounds, and by the study of the interactions of chemicals and biologicals with normal and tumor cells in vitro and with tumors and tumor cells in vivo in mice.

Areas of special interest are organic chemistry, biochemistry, medicinal chemistry, cell biology, tissue cultune, cancer biology, cancer chemotherapy, and selenium chemistry.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AM 19806-07LC

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Nature of Steroid-Receptor Interactions

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. S. Simons, Jr.	Research Chemist	NIADDK-LC
Other:	T. E. J. Robinson	Visiting Fellow	NIADDK-LC
	N. S. Lamontagne	Research Chemist	NIADDK-LC

COOPERATING UNITS (if any)

E. I. B. Thompson, NCI; H. J. Eisen, NICHD

LAB/BRANCH

Laboratory of Chemistry

SECTION

Microanalytical Services and Instrumentation

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.9	2.9	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this project is to define the initial, intra-cellular events of steroid hormone action. Synthetic glucocorticoid derivatives, some of which could react to form covalently labeled receptors via affinity labeling, are being used with glucocorticoid-responsive rat hepatoma tissue culture cells to examine: (1)steroid-receptor binding site interactions; (2)the effects of steroid binding on receptor conformation; and (3)the nature of "activation" of receptor-steroid complexes. One these synthetic steroids (dexamethasone 21-mesylate) has been patented as the first irreversible antiglucocorticoid. Using antibodies to rat liver glucocorticoid receptors, we have shown that dexamethasone 21-mesylate covalently labels the receptor in the steroid binding site. A comparison of the steroid mediated events in closely related cell lines has also been used to study steroid hormone action. With this approach, evidence for a new level of complexity in steroid hormone action has been obtained.

PHS-6040
(Rev. 2-81)

ANNUAL REPORT OF THE
LABORATORY OF EXPERIMENTAL PATHOLOGY

In the Section of Macromolecular Genetics, a number of viruses are being studied including Adenovirus, which causes malignant transformation of cells, and the adenoassociated virus, which inhibits this transformation. Low density, non-infectious adenoassociated virus particles have been isolated which contain genomes with deleted internal regions or cross linked DNA. Cloning of these variants may yield information on replication and recombination mechanisms in these viruses. Certain of the defective adenoassociated viruses are non-the-less able to inhibit formation of tumors induced by human adenovirus type 12 in Syrian Hamsters. Those defective adenoassociated viruses highly enriched for the DNA of the terminal regions were the most effective, indicating some relationship between that genome region and inhibition of adenovirus oncogenicity.

The nature of the helper function of adenovirus on adenoassociated virus has also been studied. A special part of the adenovirus early gene region 1 may make a product that protects AAV DNA from a DNAse made by adenovirus. A number of mutants in region I of AD do not promote growth of AAV. This system is reminiscent of the bacterial rec mutants. Another aspect of the analysis of how AD helps AAV has been the finding that a site in the vacinity of the early region 4 genes in AD is required for the conversion of the infecting AAV parental single strand to a double strand RF molecule.

Further studies on AAV DNA replication have involved detection of different types of nucleosome structure using staphlococcal nuclease. It seems that the structure varies with the time after infection and is not dependent on helper virus. An analysis of polyA(rich) mRNA from infected cells has lead to the conclusion that interference by defective interfering genomes occur mostly at the level of DNA replication rather than transcription.

Studies on cytogenetics of hematopoetic tissue, and on congenital and developmental disorders continue in the Section on Cytogenetics. Bone marrow cells transfused into recipients in different states of marrow suppresion revealed a even distribution of donor cells in the marrow regardless of the number of stem cells already there. It was also found that transfusion of syngeneic male donor cells into female receipients was much more successful than transfusion of female donor cells into males. An investigation into the possible hormonal basis of this difference is in progress. Finally, the susceptibility of radiation chimeras to repopulation of immunologically important tissues has been studied using NZB or DBA/2 mice.

Genetic disorders in humans have also been studied. One such disorder involves males with unusual facies, macroorchidisin and mental retardation, characterized by the so-called "fragile X" chromosome. Another disorder, involving patients with aniridia, Wilms tumor and mental retardation, has been found to be associated with a deletion of the short arm of chromosome 11 and a reduction in LDH-1. Finally, it was found that in familial and sporadic Alzheimer's disease increased numbers of metaphases with extra or missing chromosomes were not observed.

B lymphocytes from normal mice were found to respond to anti-u immunoglobulin antibodies by undergoing an enlargement over the first 24 hours and then transit through the cell cycle. By contrast mice with the xid defect were unable to enter the S phase, but did undergo size enlargement. Further studies on murine autoimmune strains NZB, MRL/lpr, and BXSB showed that MRL/lpr and NZB failed to respond to anti-u, indicating an abnormality in these two models of autoimmunity.

The New Zealand Obese (NZO) mouse has also been studied as a model of diabetes associated with autoimmunity. These mice have an increased number of tetraploid cells in the pancreas, but insulin treatment reduced the number of activated cells in both the spleen and pancreas. The possible relationship of this immunosuppressive effect of insulin to the physiology of diabetes treatment remains to be elucidated.

In the section on Cellular Function and Ultrastructure, studies on distribution of catecholamine-o-methyl transferase in rat uterus continue, and new studies on changes in tight junctions in response to mechanical stretch have been carried out. Acute mechanical stretch can lead to breakage of junction segments, as in cells of rat aorta and periplaneta mid gut. By contrast, only stretched junctional regions and prominant attached cytoskeletal elements elements are observed in isoproterenol-stimulated rat parotid gland. Studies in this section are also under way on mechanical properties of artitular cartilage and on the possibility that conventional designs of the surface of artificial femur heads may be defective. Indeed, a dimpled pattern rather than a plain surface may e better in terms of synovial fluid lubrication.

Work on the chemistry and structure of myloid and amyloid-related proteins have been continued in the Section on Molecular Pathology. Two patients having polyneuropathy, hypernephroma and a Lamda-type amyloid have been studied. Biopsy material was extracted and amyloid fibrils isolated and purfied by gel filtration. Such patients may represent a novel and distinct syndrome. Another clinical type of hereditary amyloidosis involving a rare urethral amyloidosis has been detected in the son of a father of Ashkenazai Jewish origins and a mother of American Indian descent. Finally, cerebrovascular amyloidosis (congophilic angiopathy) has been found to occur in at least 89% of all patients with Alzheimer's Disease. The nature of the amyloid material is now under investigation.

Work on the dye, Lucifer Yellow and its application to the optical analysis of nerve cell shape in mulloscan neurons has been continued in the Section on Physical Histology. Attempts also continue to synthesize a red rhodamine dye to provide a contrast with Lucifer Yellow for intracellular injection. Finally, genetic analysis of nerve cell shape in a fresh water snail remain in progress.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 21,001-28 LEP

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Preparation of stained tissue sections for investigation and diagnostic purposes.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Virginia Reveal Head, Tissue Preparation Lab. LEP NIADDK

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Experimental Pathology

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 1	PROFESSIONAL: 0	OTHER: 1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The tissue laboratory will continue to provide technical assistance to the investigators of the Laboratory of Experimental Pathology and to those of other units at the NIH who require technical help. A considerable part of the tissue laboratory's work is concerned with providing a wide variety of special histologic techniques adjusted to the various research projects as shown below.

	Specimen Accessioned	Stained slides		Spare Slides	Total
		Routine	Special		
Animals	137	290	550	4980	
Human	5	4		4	
	142	294	550		
		844		4984	
					5828

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 21,002-17 LEP

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Cellular Function and Ultrastructure

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Lois W. Tice	Chief, Section on Cellular Function and Ultrastructure	LEP	NIADDK
OTHER:	Raymond Carter	Biomedical Technician	LEP	NIADDK
	Marjorie Cahill	Bio. Lab.	LEP	NIADDK
	Asima Majumder	Visiting Fellow	LEP	NIADDK

COOPERATING UNITS (if any)

Dr. C.R. Creveling, LC:NIADDK; Dr. A.H. Hand, NIDR: Dr. D.S. Smith, Dept. of Zoology, Oxford University, England; Dr. K. Inoue, Chiba, Japan

LAB/BRANCH
Laboratory of Experimental Pathology

SECTION
Section on Cellular Function and Ultrastructure

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 4	PROFESSIONAL: 2	OTHER: 2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Studies in the changes in tight junctions in response to mechanical stretch were carried out. Studies on the distribution of COMT in rat uterus were continued.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 21,003-14 LEP

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Biomechanics and related studies.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C.W. McCutchen	Research Physicist	LEP	NIADDK

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Experimental Pathology

SECTION
Section on Cellular Function and Ultrastructure

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0.5	PROFESSIONAL: 0.5	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Attempts to determine if swelling pressure prestresses the collagen fibers in cartilage, so that a finite mechanical load on cartilage is required before it even starts to get compressed, have been inconclusive.

A simple tracer experiment suggests that when a prosthetic femur head is used with a natural acetabulum the nutrition anl lubrication of the latter's cartilage can be improved by dimpling the femur head like a golf ball.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 21, 005 -15 LEP

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)
Histochemistry: Principles, Methods & Applications

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Ned Feder	Chief, Section on Physical Histology	LEP	NIADDK
	Walter Stewart	Research Physicist	LEP	NIADDK

COOPERATING UNITS (if any)
Dr. Raymond F. Chen, NHLBI

LAB/BRANCH
Laboratory of Experimental Pathology

SECTION
Section on Biophysical Histology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5	3	2

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER
☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Continuing studies with the dye, Lucifer Yellow, have been carried out.

Studies have continued on the genetics of nerve cell shape using the snail Biomphalaria glabrata as an experimental animal.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 21,006-14 LEP

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Protein and proteolytic enzyme interrelationships in normal and disease states.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Glenner, G.G.	Chief, Section on Molecular Pathology (Visiting Scholar, Dept. of Medicine, UCSD, La Jolla, CA)	LEP	NIADDK

COOPERATING UNITS (if any)
Dr. D.J. Selkoe, Harvard; Dr. J.E. Seegmiller, UCSD; Dr. W.K. Engel, USC, LA, CA; Dr. P. Lampert, UCSD, PATH.; Dr. S. Jujihara, Yamaguchi, Japan; Dr. M.C. Dalakio, N:MN; Dr. E.D. Eanes, D:LBS.

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Laboratory of Experimental Pathology

SECTION
Section on Molecular Pathology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

It is the purpose of this project to study the chemistry and structure of amyloid and amyloid related proteins. The main topics of present interest are:

1. Isolation and chemical characterization of amyloid fibril protein from various types of systemic amyloidosis and localized amyloid deposits.
2. Isolation and physical and chemical characterization of the paired helical filaments of the neurofibrillary tangles and the amyloid fibrils of the neuritic plaques and cerebral vessels in Alzheimer's disease.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 21, 008-16 LEP

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Cytogenetics

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Tjio, H.H.	Chief, Section on Cytogenetics	LEP	NIADDK
OTHER:	White, B.J.	Medical Officer (Research)	LEP	NIADDK
	Raveche, E.S.	Research Chemist	LEP	NIADDK

COOPERATING UNITS (if any)
C. Brecher, University of California, Berkeley, California; A. Steinberg, AR:NIADDK.

LAB/BRANCH
Laboratory of Experimental Pathology

SECTION
Section on Cytogenetics

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 2	PROFESSIONAL: 1	OTHER: 1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

1. Application of differential staining techniques and continued improvement of established methods are basic to all of the projects conducted in the laboratory.

2. Human cytogenetic studies of patients with congenital and developmental disorders as well as studies of mutagen-induced chromosomal abnormalities are continuing.

3. Murine cytogenetic studies include evaluation of mitotic and meiotic chromosomes and karytypic evaluation of cells in hematopoetic tissue regeneration experiments. These are part of projects utilizing the mouse as a model for human diseases.

4. Correlation of flow cytometric analysis with cytogenetic findings.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 21,009-15 LEP

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Cytogenetic Studies

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	White, B.J.	Research Medical Officer	LEP	NIADDK
OTHER:	Tjio, J.H.	Chief, Section on Cytogenetics	LEP	NIADDK

COOPERATING UNITS (if any)
S. Rosen, CE:NIADDK; E.T. Bersu, Dept. of Anatomy, Univ. of Wisconsin-Madison; M.Kaiser, CB:NEI; Inter-Institutes Genetics Unit, NIH; S. Rapoport, LN:NIA; N. Cutler, LN:NIA.

LAB/BRANCH
Laboratory of Experimental Pathology

SECTION
Section on Cytogenetics

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	1	1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

1. Studies of patients and their families with certain genetic disorders due to chromosomal abnormalities or single gene defects. Techniques used include high resolution chromosomal banding and differential staining, analysis of genetic markers such as enzymes or HLA within pedigrees, and dermatoglyphic studies. Findings are correlated with phenotypic abnormalities and may be used for genetic counseling.

2. Karyotypic evaluation of tissues from laboratory animals which are models of different human genetic disorders, primarily primate and murine. Relationship of cytogenetic variation to defects associated with certain diseases is stressed. Effects of gross chromosomal abnormality upon reproductive fitness and growth and development are studied.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 21, 010-06 LEP

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Function of DNA virus genomes in animal cells.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Barrie J. Carter	Chief, Section on Macromolecular Genetics	LEP	NIADDK
OTHER:	Carol J. Marcus	Senior Staff Fellow	LEP	NIADDK
	Periannan Senapathy	Visiting Fellow	LEP	NIADDK
	Anne Colberg-Poley	Guest Worker	LEP	NIADDK
	Stephanie Seidman	Staff Fellow	LEP	NIADDK
	Michael H.P. West	Visiting Associate	LEP	NIADDK
	Jon-Duri Tratschin	Guest Worker	LEP	NIADDK
	Irving Miller	Biologist	LEP	NIADDK
	Catherine A. Laughlin	Guest Worker	LEP	NIADDK

COOPERATING UNITS (if any)
N. Jones, Purdue; G. Ketner, Johns Hopkins; L. Enquist, R. Watson, Molecular Genetics Inc., Minnesota; S. Bear, D. Court, NCI, C. Laughlin USUHS.

LAB/BRANCH
Laboratory of Experimental Pathology

SECTION
Section on Macromolecular Genetics

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 6.3	PROFESSIONAL: 4.8	OTHER: 1.5

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We are employing DNA viruses as molecular probes to study genome expression in human cells. We are studying intensively the structure and function of adeno-associated virus (AAV) since this virus has only a small genome. We are studying also adenovirus which is a more complex genome. Adenovirus is the helper virus for AAV multiplication. This helper relationship is being analysed. Also both AAV and adenovirus recombine with cellular DNA. In the case of adenovirus this causes malignant transformation of the cell. AAV inhibits this transformation and also inhibits Ad12 oncogenesis in newborn animals. Thus AAV inhibits tumor induction. The mechanism of this inhibition of tumor induction is being studied at the molecular level in both cell culture and in animal experiments. We are also analysing gene expression and recombination in herpes simplex virus which is also a helper for AAV and has been linked to cerivcal carcinoma.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 21, 012-05 LEP

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)
Cytogenetics and Immunogenetics

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Raveche, E.S.	Research Chemist	LEP	NIADDK
OTHER:	Tjio, J.H.	Chief, Section on Cytogenetics	LEP	NIADDK

COOPERATING UNITS (if any)
A. Steinberg, NIADDK: A. DeFranco, NIAID: W.E. Paul, NIAID, K. Melez, Georgetown University.

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Laboratory of Experimental Pathology

SECTION
Section on Cytogenetics

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 2	PROFESSIONAL: 2	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

1. Application of differential staining techniques for chromosome analysis.
2. Studies of the inheritance of several autoimmune immunological traits including antibodies cytotoxic for T lymphocytes and reactive with single stranded DNA and chromosomal hyperdiploidy.
3. Use of flow cytometry in the analysis of aneuploid cells.
4. Sex hormone receptors in murine thymus.
5. In vivo cell cycle analysis of polyclonal B-cell activation by flow cytometric techniques.
6. In vitro analysis of autologous mixed lymphocyte reaction in normals and patients with Systemic Lupus Erythematosus.
7. The use of recombinant inbred lines to determine the presence of an underlying defect - giving rise to autoimmune disease.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM-21, 013-05 LEP

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Electron Microscopic Investigations

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Howard A. Bladen	Research Biologist	LEP	NIADDK
OTHER:	Jacqueline Henson	Histopathologist	LEP	NIADDK

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Experimental Pathology

SECTION
Laboratory of the Chief

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

INACTIVE

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 21,014-05 LEP

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Interferon inhibition of virus (s) which bud from plasma membrane

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. Maheshwari	Guest Worker	LEP	NIADDK
OTHER:	R. Friedman	Guest Worker	LEP	NIADDK

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Experimental Pathology

SECTION
Section on Chemical Pathology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

INACTIVE

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 21, 017-03 LEP

PERIOD COVERED
October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)

Biochemical Mechanisms of Interferon Action

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Dr. David A. Epstein	Staff Fellow	LEP	NIADDK
OTHER:	Dr. Christine Czarniecki	Staff Fellow	LEP	NIADDK
	Dr. Robert Friedman	Guest Worker	LEP	NIADDK
	Dr. Helmuth Jacobsen	Visiting Fellow	LEP	NIADDK

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Experimental Pathology

SECTION
Section on Chemical Pathology

INSTITUTE AND LOCATION
NIADDK, LEP, Bethesda, Maryland 20205

TOTAL MANYEARS: 0	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

INACTIVE

PHS-6040
(Rev. 2-81)

ANNUAL REPORT OF THE LABORATORY OF BIOCHEMICAL PHARMACOLOGY

I. POLYAMINES

Polyamine Biosynthesis in Escherichia coli

In previous studies we have isolated the various enzymes concerned with polyamine biosynthesis in *Escherichia coli* and have defined and mapped the genes concerned with the biosynthesis of these enzymes. We have constructed mutants deficient in each of these steps and have prepared strains that cannot synthesize polyamines. The purpose of these studies is to find what the action of polyamines is *in vivo*, i.e., the physiological role of the polyamines.

In our more recent studies we have found that these mutants are defective in one phase of protein biosynthesis *in vivo*; namely, translation. We have shown that the insertion of a *rpsL* (*strA*) mutation into the polyamine-deficient strain converts the strain from a slow-growing organism into a strain that will not grow in the absence of added polyamines. The *rpsL* mutation is known to affect the S12 ribosomal protein, and these results indicate that the polyamines have an effect on the protein-synthesizing ribosomal complex.

More direct evidence for an effect of polyamines on translation in protein biosynthesis *in vivo* has been obtained by showing a defect in the multiplication of amber bacteriophages T4 and T7 in these polyamine-deficient cells, even though they contain a suppressor gene; the suppressor gene permits these amber phage to multiply well if polyamines are added. Wild type bacteriophage T4 and T7 multiply well in the presence or absence of polyamines. Thus, there is a marked difference in the multiplication of wild type and amber bacteriophages T4 and T7 in polyamine-deficient cells. By measuring the proteins formed after infection of amine-deficient and amine-supplemented strains with gene 1^-*am*342 bacteriophage T7 and wild type bacteriophage T7, we have been able to show the accumulation of an amber fragment of the gene 1 protein product, confirming that there is a defect in the reading of the amber codon in the absence of the amines. The most likely explanation is an effect of polyamines on the stability or conformation of the protein-synthesizing ribosomal complex.

In other studies in *E. coli* we have continued our work on the plasmid that contains the gene responsible for adenosylmethionine decarboxylase. By standard restriction enzyme treatment, we have decreased the size of the insert to a size suitable for DNA sequencing. The reason for this study is the interesting cofactor (covalently-bound pyruvate) that we have shown is required for the activity of this enzyme and the postulation that the active decarboxylase is formed from a precursor form. We would like to sequence the genes for this enzyme, since the enzyme is probably first formed as a larger proenzyme; the active enzyme contains covalently-bound pyruvate at the amino terminus, and we postulate that this is formed by cleavage of the precursor form.

. . . . . Dr. H. Tabor and Dr. C. W. Tabor (LBP, NIADDK)

Purification of Ornithine Decarboxylase of Saccharomyces cerevisiae. Control of Polyamine Biosynthesis in Yeast

We have continued our studies on the purification and regulation of ornithine decarboxylase of *S. cerevisiae*, the rate-limiting enzyme in the biosynthesis of putrescine, spermidine, and spermine. We have prepared an antibody column, which permits us to obtain 70% pure enzyme in one step. We have also developed immunological methods that permit us to study an enzyme in a crude fresh extract without proteolysis. In this procedure the enzyme is labeled in vivo with [^{35}S]methionine, immunoprecipitated from a fresh crude extract, and examined by radioautography after electrophoresis. We have now found evidence that the enzyme obtained by this technique differs in size from the enzyme which we had purified by standard techniques. The new band (86,000) is converted with time at 4°C to 82,000 and then to a stable form, 68,000 molecular weight. The changes result from proteolysis, since addition of a proteolytic inhibitor markedly slows the conversion.

We have shown that our mutants (spe2) with high enzyme activity, our mutants (spe10) lacking ornithine decarboxylase, cells which have lost their ornithine decarboxylase activity as a result of growth with amines, and wild type cells with very low activity, all have equal amounts of immunoprecipitable protein in the 86,000 position. Thus, we assume that post-translational modification of this enzyme accounts for changes in enzyme activity. The conversion from 85,000 to 68,000 molecular weight is not related to the post-translational modification.

. . . . . Dr. C. W. Tabor and Drs. H. Tabor and A. K. Tyagi (LBP, NIADDK)

II. MECHANISMS OF INHERITANCE IN SACCHAROMYCES CEREVISIAE

The Killer Virus-Plasmid of Yeast

Background. Killer strains of yeast are those secreting one of two protein toxins coded by one of two double-stranded RNAs. M_1 ds RNA encodes the K_1 toxin, and M_2 ds RNA encodes the K_2 toxin. Most strains (killer or not) carry another ds RNA, called L, which encodes the major coat protein in which all ds RNA molecules are found as virus-like particles (VLPs). We have previously described 3 chromosomal genes whose products are essential for expression of information in M_1 ds RNA (kex and rex genes), 29 chromosomal genes needed for maintenance or replication of M_1 (27 mak genes, pet18, and spe2), and 6 genes with regulatory effects on K_1 plasmid replication (4 ski genes, KRB1, and a mitochondrial gene). We have also discovered a replication gene (sd) located on the killer plasmid M_1 ds RNA. A new plasmid, called [EXL], eliminates M_2 ds RNA when both are introduced into the same cell. A second new plasmid, [NEX], prevents [EXL] from eliminating M_2 ds RNA. Surprisingly, M_2 ds RNA needs a new chromosomal gene, MKT1, only if [NEX] is present. A third cytoplasmic gene, called [HOK], was discovered, based on its ability to support the replication-defective mutant of M_1, called [KIL-sd$_1$]. We showed [HOK] and [NEX] are on a form of L ds RNA. The presence of a second form of L ds RNA in the same strains was inferred.

New Findings.

Multiple L ds RNAs. Yeast strains can carry one or more of at least three very different L ds RNAs, called L-A, L-B, and L-C. Variants of L-A carry combinations of the non-Mendelian genes [HOK], [NEX], and [EXL]. L-A needs, for its replication, the products of MAK3, MAK10, and PET18, while L-B and L-C do not. L-A is eliminated by growing cells at 37-39°C; L-B and L-C are not. L-A codes for a 81,000 dalton major virus-like particle (VLP) protein. L-B and L-C, in strains lacking L-A, are also present in VLPs with RNA polymerase activity, but these VLPs lack the 81,000 and 180,000 dalton proteins present in L-A-containing VLPs. Their major proteins are 77,000 and 73,000 daltons.

. . . . . Drs. S. S. Sommer and R. B. Wickner

Ribosomal Protein L3 Is Involved in M_1 and M_2 ds RNA Replication. The MAK8 gene is identical to the TCM1 gene, the latter first identified as a locus determining trichodermin resistance and later shown to code for ribosomal protein L3. All tcml mutants lose the K_1 or K_2 genomes (M_1 and M_2 ds RNAs), but not any of the forms of L ds RNA.

. . . . . Drs. R. B. Wickner, S. P. Ridley, and S. G. Ball

Mutants in *ski2, ski3,* and *ski4* Suppress *mkt1* and Cause Cold-Sensitivity for Growth. ski2 mktl double mutants stably maintain K_2 even if NEX is present (unlike SKI^+ mktl strains). The same is true for ski3 mktl and ski4 mktl, but not for skil mktl strains. Mutants in ski2, ski3, or ski4 are cold-sensitive for growth, but only if they carry a killer plasmid. A [KIL-sd_1] mutant killer plasmid makes these strains only slightly cold-sensitive.

. . . . . Drs. S. P. Ridley, S. S. Sommer, and R. B. Wickner

Unusual Bases in Double-Stranded RNA. L and M ds RNAs have two modified nucleotides, one of which migrates near 5'-CMP. The other is probably a modified adenine.

. . . . . Drs. S. S. Sommer and R. B. Wickner

III. NUCLEIC ACIDS

Small Nuclear RNA Genes of the Rat

In our attempt to identify genes that must be expressed to attain or maintain a particular state of cellular differentiation, we have compared the types of small nuclear RNAs (snRNAs) synthesized in various normal and transformed rat cells. As judged by polyacrylamide gel electrophoresis, we found that our rat tumor cell line contained the major types and pattern of snRNAs described previously by others (e.g., the species designated U1, U2, U3, U4, U5, U6, 5S, and several not named). However, we found differences among the patterns of the snRNAs present in muscle, kidney, liver, and the tumor cell line. We found these differences not only for the previously described, typical snRNA species but also for those newly discovered by us. These differences are also seen when nuclei are prepared from normal cells in the presence of vanadium ribonucleoside complex, a powerful ribonuclease inhibitor. We are current characterizing several of the more dramatic differences that we have found, which include: the presence in kidney cells of a U2 molecule one-half the length of the normal U2; the almost complete absence in muscle nuclei of most of the small nuclear RNA species except for U1; the very large amounts relative to U1 of U4, U5, and U6 in normal liver cells. We have shown that whole kidney nuclei can cleave exogenously added U2 molecules to a form that is exactly like the one from kidney nuclei. We only observed this when the U2 molecules are added as ribonucleoprotein particles. We are currently preparing cDNA clones of the snRNAs so that we can confirm by hybridization the differences in the snRNA patterns described and determine whether the "short" U2 snRNA species present in kidney nuclei is present in intact nuclei or is produced after cell breakage.

. . . . . Drs. E. Csordas-Toth and A. V. Furano

Repetitive DNA Elements in the Rat

Approximately one-third of the mass of rat (Rattus norvegicus) DNA is comprised of an undetermined number of families of repeat DNA. Only one family, consisting of about 100,000 93 base pair (bp) elements arranged in blocks of tandem arrays located in centromeric and telomeric chromosomal regions, has been characterized.

Ancestral rodent repeat families, i.e., those present in both Rattus and non-Rattus genera, are more likely to be transcribed than are the repeat families specific to

Rattus. Almost 40% (29 of 76) of ancestral rat repeat families are transcribed in rat tissues, whereas only 10% of about 400 randomly selected Rattus specific families are transcribed. Of the 13 families that are transcribed in all 4 different rat cell types examined, 8 (or 60%) are present in non-Rattus genomes. The sequence and organization of these repeat DNA elements and their flanking DNA sequences within the various rodent genomes is now being examined.

A number of the new repeat families, i.e., those found only in Rattus, may be related to each other by virtue of their homology to the 93 bp high-repeat family referred to earlier. Neither the 93 bp family nor any members of its superfamily are transcribed. Furthermore, the 93 bp family is probably undergoing change in present day Rattus. First, the 93 bp family of R. norvegicus contains a large number of minor sequence variants. Each of the minor sequence variants is arranged in a periodic fashion in one or more blocks of the tandem arrays. Thus as a variant sequence increases in number, it enters only certain locations within a tandem array. This and other features of variant sequence evolution is under investigation.

Second, we have found that Rattus rattus, the only other species of the genus Rattus, contains a version of the 93 bp repeat that is not present in R. norvegicus. This R. rattus version accounts for about 80% of the 93 bp elements in R. rattus, and the remaining 20% are very similar to those present in R. norvegicus. Furthermore, R. rattus seems to contain the entire repetoire of all the other repeat DNA families that we found in R. norvegicus. The R. rattus specific 93 bp variant does not coexist with the R. norvegicus type in the same tandem array. This substitution has not taken place in a laboratory strain of R. norvegicus (e.g., Sprague-Dawley) or in an R. norvegicus tumor line, the Jensen sarcoma, which has been propagated as a tumor since 1907.

. . . . . Drs. F. R. Witney, D. A. Epstein, and A. V. Furano

The Requirement for Two Active tuf Genes in E. coli

We have recently completed our studies on this project; we constructed several isogenic strains that differ only by containing an inactive tufB gene. The parent strains containing an active tufB gene contains an electrophoretic variant of the tufA gene, and thus we are able to measure the relative amounts of tufA and tufB gene product in the parental strain as well as assess the removal of the tufB gene product on synthesis of the tufA product in the tufB$^-$ strain. In agreement with our earlier results we found that inactivation of the tufB gene causes a compensatory increase in the synthesis of the tufA gene product which is brought about by a derepression of the operon in which the tufA gene resides. More interestingly, we find that the tufB$^-$ cells exhibit a long lag before they shift up their growth rate when their growth media is nutritionally enriched. This is not due to the trivial reason that they cannot make sufficient EF-Tu from a single tuf gene fast enough after the nutritional shift up because the lag before shift up is less for a very rich nutritional enrichment than for a poorer nutritional enrichment. Our data indicates that the lag in shift up is related to the cell's metabolic adjustments necessitated by growth with only one tuf gene. Regardless of the mechanism, the fact that cells with only one tuf gene are unable to immediately take advantage of a nutritional enrichment puts them at a marked selective disadvantage to wild type cells and could rationalize the widespread occurrence of duplicate tuf gene in many prokaryotic genera.

. . . . . Drs. F. S. Young and A. V. Furano

DNA Replication: The Bacteriophage T4 System

We are studying the replication of duplex DNA using proteins produced by bacteriophage T4. At present this system consists of seven phage-encoded proteins which efficiently catalyze the reactions required for continuous strand displacement DNA synthesis on the leading strand and for initiation and elongation of new chains during discontinuous synthesis of the complementary lagging strand. Each of these proteins has been purified to near homogeneity, using appropriate enzymatic or complementation assays.

Primer Synthesis

The gene 41 and gene 61 priming proteins synthesize pentanucleotides with the sequence pppACNNN using single-stranded DNA templates. 61 Protein is a very basic protein with a molecular weight of 44,000 daltons, which is active as a monomer. During the past year we have shown that for primer synthesis there is a linear dependence on 61 protein concentration, but a sigmoidal dependence on 41 protein concentration. The dependence on 41 protein concentration is linear, however, if the 41 protein is first "activated" by incubation, at high concentration, in the presence of rGTP. Using this "activated" 41 protein and purified 61 protein, there is a stoichiometric relationship between the two proteins in the priming reaction, consistent with the existence of a priming complex comprising an oligomer of 41 protein and a 61 protein monomer.

. . . . . Drs. L. L. Silver and N. G. Nossal

DNA Unwinding (Helicase) Activity

We have recently shown the gene 41 priming protein is also a DNA helicase which unwinds short fragments annealed to single-stranded DNA in a GTP- or ATP-dependent reaction, beginning at the 3' terminus of the fragment. 41 Protein helicase activity is stimulated by the T4 gene 61 priming protein and by the T4 gene 44/62 and 45 polymerase accessory proteins, but is strongly inhibited by the T4 gene 32 DNA binding protein. Using singly nicked circular duplex DNA templates, we find that 41 protein markedly increases the rate at which chains are elongated by T4 DNA polymerase and the T4 gene 44/62, 45, and 32 proteins. These results support a model in which 41 protein moves 5' to 3' along the template for the lagging strand in search of the next available site for primer synthesis, and in this process helps to unwind the template for the leading strand in a 3' to 5' direction.

. . . . . Drs. N. G. Nossal, M. Venkatesan, and L. L. Silver

Polymerase Accessory Proteins

The polymerase accessory proteins, which consist of the T4 gene 45 protein and the complex of the gene 44 and 62 proteins, increase the rate and processivity of synthesis on primed single-stranded templates in an ATP-dependent reaction. We have previously shown that these proteins facilitate the movement of the polymerase through kinetic barriers or "pause sites" created by secondary structure in the template and decrease the dissociation of the polymerase from the DNA template. Addition of T4 gene 32 helix-destabilizing protein eliminates the barriers, increases the rate of synthesis, and reduces nucleotide turnover. We now find that the accessory proteins and gene 32 protein also stimulate hydrolysis of duplex DNA by the 3' to 5' exonuclease activity of the polymerase. This stimulation of hydrolysis by the 44/62 and 45 proteins is dependent on both ATP and the presence of a protruding single strand of DNA which apparently serves as a binding site for one or more of the accessory proteins. The accessory proteins do not stimulate duplex DNA hydrolysis by the B22 nuclease, an amber fragment made up of the N-terminal 80% of the wild type polymerase.

. . . . . Drs. M. Venkatesan and N. G. Nossal

Evolution

The capacity of nucleotide bases to form hydrogen bonds with peptides has been further investigated with the interaction of cytosine with peptides of serine and threonine the central point of interest. This interaction, previously demonstrated in organic solvents, has now been shown to occur also in water when suitable derivatives are used.

. . . . . Drs. C.-H. Niu and S. Black

IV. COMPLEX CARBOHYDRATES

Many monoclonal antibodies produced by hybridomas obtained from mice immunized with human tumors are directed against glycolipids. These glycolipid antigens can be detected on thin-layer chromatograms of total lipid extracts of tissues by application of antibody followed by ^{125}I-labeled rabbit anti-mouse IgG and autoradiography. Using this technique, two antibodies were found to be directed against a monosialoganglioside not found in normal adult tissue whose carbohydrate structure is NeuNAcα2-3Galβ1-3[Fucα1-4]GlcNAcβ1-3Galβ1-4Glc. The ganglioside occurs in fetal tissue, suggesting that the antibodies recognize an "onco-fetal antigen." The antibodies detect antigen in sera from most patients with colorectal, gastric, and pancreatic adenocarcinomas. The antigen in serum appears to be mucin, not a ganglioside.

Over 100 monoclonal antibodies, reported to be more or less specific for various cancers, have been obtained from several investigators. About one third of these have been found to be directed against a sugar sequence found in the oligosaccharide lacto-N-fucopentaose III, Galβ1-4[Fucα1-3]GlcNAcβ1-3Galβ1-4Glc. The sequence must be very immunogenic in mice. Interestingly, all antibodies with this specificity thus far examined are of the immunoglobulin M type.

. . . . . Drs. J. L. Magnani, M. B. Brockhaus, L. C. Huang, and V. Ginsburg

V. MEMBRANE STUDIES OF MACROPHAGES AND OF ESCHERICHIA COLI

Complement Activation by Lipopolysaccharide (LPS) Determines Phagocytosis of Salmonellae by Macrophages and Their Virulence in Mice

S. typhimurium becomes less virulent for mice when genes which specify abequose in its LPS O-antigen polysaccharide repeating unit are replaced by ones specifying tyvelose, and even less virulent when the entire repeating unit is replaced with one of a different structure. We have now demonstrated differential activation of complement, via the alternative pathway, by the different polysaccharides: the most virulent activates the complement protein C3 the least, and the least virulent activates it the most. The resultant differential deposition of C3b on the bacteria determines their ingestion via the C3b receptor of phagocytic cells. Thus, macrophages in vitro ingest the most virulent bacterium at the slowest rate, the one of intermediate virulence faster, and the least virulent fastest. This mechanism operates in vivo: the least virulent bacteria are cleared much faster from the blood of mice than the most virulent; further, destroying complement with cobra venom factor slows clearance of the least virulent bacterium but not the already low clearance of the most virulent. These findings demonstrate for the first time that slight changes in polysaccharide repeating unit composition of

LPS can determine complement activation, phagocytosis, and virulence in the non-immune host.

. . . . . Drs. L. Leive, N. Grossman, and P. H. Makela, and C.-J. Liang-Takasaki

Studying Phagocytosis in Macrophages by Mutation and Induction

(1) Selection of Mutants Deficient in General Phagocytosis. The metabolic steps in phagocytosis, and the biochemical differences between receptor-mediated and "general" (receptor-independent) phagocytosis, are obscure. Mutants in phagocytosis would help define these pathways, and other laboratories have isolated some mutants lacking receptor-mediated phagocytosis but normal in general phagocytosis. To isolate mutants deficient in general phagocytosis, we derivatized polystyrene beads with the toxin daunomycin and fed them to the murine macrophage-like cell line J774; all cells were killed. Mutagenized cells were exposed to beads, and survivors were selected from a dozen separate mutageneses. The isolated new lines could phagocytose 20%-70% as fast as the parent strain, as measured by uptake of beads derivatized with labeled glycine instead of daunomycin; thus their survival reflected impaired phagocytosis and not resistance to the toxin. All showed normal uptake via the Fc receptor, emphasizing the differences in pathway between receptor-mediated and general phagocytosis. These lines provide a new tool to dissect the biochemical steps in general phagocytosis.

. . . . . Drs. S. Takasaki and L. Leive

(2) Induction of Fc-Mediated Phagocytosis by DMSO. Another murine macrophage-like cell line, P388D1, can carry out general phagocytosis and has Fc receptors, but cannot carry out Fc-mediated phagocytosis. We now find that exposure to DMSO (1.5% for 48 hours) induces receptor-mediated phagocytosis in these cells as indicated by ingestion of antibody-coated rbc, without affecting general phagocytosis or apparent number of Fc receptors. No slowing of growth nor alteration in lysosomal enzymes is observed until much longer exposure. DMSO removal results in prompt loss of receptor-mediated phagocytosis, so events during both induction and loss can be studied. This system will be of value in studying steps in receptor-mediated phagocytosis.

. . . . . Drs. S. Takasaki and L. Leive

Structure, Metabolism, and Function in the Outer Membrane of Gram-Negative Bacteria

(1) Control of O-Antigen Synthesis. LPS consists of a lipid that forms the outer leaflet of the outer membrane and a core polysaccharide substituted by O-antigen repeating units. We previously showed, using sodium dodecylsulfate gel chromatography, that lengths of O-antigenic polysaccharide in a given strain vary from 0 to more than 45 units, and that the distribution of sizes is not uniform. We have now used mutants of S. typhimurium partially blocked in the synthesis of O-antigen to study the regulation of chain length. As expected, the average chain length is much lower in the mutant than in the wild type, and the percent of LPS molecules with 0 or 1 unit went up to 74% in the mutant compared to 35% in the wild type. However, the mutant still continued to synthesize LPS molecules with a range of 0 to more than 40 subunits. These and other data indicate that the enzymes responsible for polymerization or transfer of O-antigen units show a preference for certain lengths.

. . . . . Drs. R. C. Goldman and L. Leive

(2) Characterization of a New "Capsular-like" Polysaccharide on the Surface of Certain E. coli Strains. Several medically important strains of E. coli reacted immunologically as though they had a capsule, but none could be found. We now have shown that these strains contain a polymer, consisting of very long O-antigen chains not linked to lipid A (and therefore not LPS) outside the cell. The size, composition, and location of this polymer has been determined, and it has been shown to cause the above anomolous capsule response.

. . . . . Drs. R. C. Goldman and L. Leive

(3) Permeability of the Outer Membrane. The outer membrane is impermeable to hydrophobic molecules, in large part because of LPS, but the contribution of associated proteins is unclear. We have devised a test for hydrophobic permeability using tetracyclines of varying hydrophobicity and find that mutants lacking the ompA major outer membrane protein show increased permeability to hydrophobic molecules; loss of some other major outer membrane proteins has no effect. This finding provides the first reasonable primary function for this protein.

. . . . . Drs. L. Leive and J. D. Foulds (LBM, NIADDK)

Aldoheptose Biosynthesis and Its Regulation

A mutant of Escherichia coli K-12 carrying a single site mutation, designated rfaD, which affects the synthesis of the aldoheptose, L-glycero-D-mannoheptose, has been isolated. Phenotypically this mutant is hypersensitive to hydrophobic drugs and the lipopolysaccharide core biosynthesis of this mutant is altered. It was observed that lipopolysaccharide of the rfaD mutant contained the aldoheptose D-glycero-D-mannoheptose rather than the normal L-glycero-D-mannoheptose. It is postulated that L-glycero-D-mannoheptose is formed from D-glycero-D-mannoheptose via a racemization.

We now find the accumulation of an unusual nucleotide, ADP-D-glycero-D-mannoheptose, in rfaD mutants. Further, this nucleotide could serve as substrate for an epimerase activity (e.g., ADP-L-glycero-D-mannoheptose-6-epimerase) present in wild type and not in rfaD mutant, as demonstrated in vitro by coupling the epimerase activity with subsequent incorporation of heptose and labeled glucose in lipopolysaccharide.

We also have identified two colicin El hybrid plasmids carrying the coding sequence of the rfaD locus; these complement the rfaD phenotype and amplify the epimerase activity. In vitro recombination techniques were used to subclone a 9-kilobase fragment for one of the $rfaD^+$ colicin El plasmid into pBR322. Using the maxicell technique we were able to identify two plasmid-coded proteins unique to the 9-kilobase insert. A partial physical map of the 9-kilobase insert has been developed.

We are now isolating and characterizing the rfaD gene product to further understand the enzyme's role and regulation in L-glycero-D-mannoheptose biosynthesis.

. . . . . Dr. W. G. Coleman, Jr.

VI. ENZYME MECHANISMS AND PROTEIN STRUCTURE

Tryptophan Synthase of Escherichia coli

Tryptophan synthase of E. coli is composed of two α and two β subunits. Each of these subunits is composed of two domains. The relationship between the domain structure of the α subunit and the mechanism of the unfolding of the α subunit has been investigated. This study utilizes two proteolytic fragments of the α subunit, α-1 and α-2, which have been shown to refold independently and to correspond to two domains of the α subunit. The effects of guanidine hydrochloride concentration on the separate α-1 and α-2 fragments, on the intact α subunit, and on the derivative nicked by trypsin (α'), are compared by measuring ellipticity at 222 nm and by measuring the susceptibility of tyrosyl residues to chemical modification. The results show that guanidine hydrochloride-induced unfolding of the α subunit results from the stepwise unfolding of the two domains: the α-2 fragment and the corresponding domain in the intact α subunit are unfolded by low concentrations of guanidine hydrochloride; the α-1 fragment and the corresponding domain in the intact α subunit are unfolded by higher concentrations of guanidine hydrochloride. Tyrosyl residues important for enzyme activity are located in the domain interaction site. Experiments with α subunit and α-1 and α-2 fragments from several mutant α proteins and from Salmonella typhimurium are probing the effect of protein sequence on the stability of the α subunit and its constituent domains.

. . . . . Dr. E. W. Miles and Drs. K. Yutani and K. Ogasahara (Institute for Protein Research, Osaka University, Osaka, Japan)

The site of interaction between the α and β_2 subunits of tryptophan synthase has been probed by differential reductive methylation studies. These studies compare the reactivity of amino groups in the separate α and β_2 subunits and in the $\alpha_2\beta_2$ complex. The most reactive amino group in the α subunit is lysine-109. Since this residue is strongly shielded from modification in the $\alpha_2\beta_2$ complex, it may be located in the subunit interaction site. The most reactive amino group in the β_2 subunit, the amino terminal threonine, is not protected from modification in the $\alpha_2\beta_2$ complex.

. . . . . Dr. E. W. Miles and Dr. Thomas Fairwell (Molecular Disease Branch, National Heart, Lung, and Blood Institute, NIH)

Chemical modification of tryptophan synthase by [^{14}C]phenylglyoxal results in the stoichiometric modification of an arginine residue in the β_2 subunit and in concomitant inactivation. Substrate protection experiments indicate that the arginine is located in the active site of the β_2 subunit. The essential arginine residue is being located in the known sequence of the β_2 subunit.

. . . . . Drs. E. W. Miles and K. Tanizawa

The pyridoxal phosphate cofactor of the β_2 subunit forms a Schiff base with a lysine residue of the protein or with the amino group of substrates. The orientation of the pyridoxal phosphate at the active site has been probed by determining the stereochemistry of the sodium borohyride reduction of the Schiff bases. Reduction in the presence of L-serine gave phosphopyridoxylalanine resulting from reduction of the intermediate Schiff base formed between pyridoxal phosphate and α-aminoacrylate. This is the first time that this intermediate has been "trapped." Analysis of the stereochemistry of the reduction of the double bond showed that it had occurred on the Si face. In contrast, analysis of phosphopyridoxyllysine obtained by hydrolysis of the reduced protein showed that the internal Schiff base double bond had been reduced on the C-4 Re face, suggesting a cofactor reorientation upon substrate binding.

. . . . . Dr. E. W. Miles and Drs. D. Houck and H. G. Floss (Department of Medicinal Chemistry and Pharmacognosy, Purdue University, West Lafayette, Indiana)

New Kinetic Model for the Gelation of Fibrin

The period of time between the addition of thrombin to a fibrinogen solution and the appearance of a rigid gel is called the gelation time. The dependence of gelation time upon initial concentrations of fibrinogen and thrombin, previously measured in this laboratory, may be semi-quantitatively accounted for by a simple kinetic model which differs in important respects from models previously proposed by others.

. . . . . Dr. A. P. Minton

Reaction Intermediates in the Gelation of Fibrin

The formation of a soluble species of approximately 1 x 10^6 molecular weight has been observed chromatographically in fibrinogen/fibrin solutions which have been retarded in their progress toward gelation by the addition of hirudin, a thrombin inhibitor. This species has been tentatively identified as a complex of two molecules of fibrinogen and one molecule of fibrin.

. . . . . Drs. J. Wilf and A. P. Minton

Effect of Macromolecular Additives upon Fibrin Gelation

It has been found that water-soluble polymers (polyethylene glycol, dextran) in relatively low concentration tend to precipitate fibrinogen from solution. In contrast, the addition of "inert" proteins (for example, hemoglbin, serum albumin, gamma globulin) at moderate concentrations does not precipitate fibrinogen, but markedly accelerates the formation of fibrin gel initiated by the addition of thrombin. These findings have significance with respect to the choice of macromolecular substances present in artificial blood substitutes and plasma expanders.

. . . . . Dr. J. Wilf

Effect of Macromolecular Additives upon Protein Stability

It has been found that the ability of stress-susceptible proteins to regain native structure and/or enzymatic activity following exposure to elevated temperature or high concentrations of ethanol is substantially affected by the addition of macromolecular additives. Whereas the addition of polymers (polyethylene glycol, dextran, ficoll) accelerates the irreversible loss of native structure and/or activity under denaturing conditions, the addition of stress-stable proteins (ribonuclease A, ovomucoid, fetuin) in many cases retards the irreversible loss of native structure and/or activity under the same conditions. A simple kinetic model accounting for the observed effects has been formulated. It has been suggested that the observed effect may account for part of the role of heat shock proteins in the acquisition of cellular tolerance to heat and ethanol.

. . . . . Dr. A. P. Minton, P. Karmin, and K. W. Minton (Department of Radiology and Pathology, Stanford University Medical Center)

Aminoacyl tRNA Synthetases

One form of the valyl tRNA synthetase of yeast can be dissociated with separation of a heat-stable, nondialyzable acidic substance that stimulates the enzyme's action. The heat-stable material has now been separated into at least four fractions, one of which has been purified to homogeneity. It is a peptide of about 18,000 molecular weight and appears to contain two residues of half cystine per mole.

. . . . . Dr. S. Black

Protein Sequences and Structure

Binding Studies and Protein Interactions. Model systems are being developed and studied for explaining cooperative effects in biological macromolecules, such as hemoglobin, collagen, clathrin, and calmodulin.

Slow Degradation of Proteins. Sterile solutions of proteins yield peptides and amino acids on standing for various periods of time. A number of systems are being studied in an attempt to explain this phenomenon adequately. It is proposed that some of the observed activity is intrinsic in the protein molecule itself.

Statistical Analyses of Protein Sequences. The concept of uniqueness and a quantitative method for its evaluation has been developed using Monte Carlo statistics. Analyses based on this concept are being used to relate isolated structures with possible biological activity. Studies so far have emphasized single species.

. . . . . Dr. H. A. Saroff

Oxidation of Oxyhemoglobin

The reactions of formaldehyde with amino acids and diamines were studied. The rates and, where possible, the equilibria for methylene bridge ring formation were determined. The slow step was one of three possibilities: (1) intermolecular reaction of formaldehyde with amino acid; (2) removal of hydroxyl from the product of this reaction; or (3) ring closure. The rate of the ring closure step varies by more than a billion from phenylalanine to some of the other amino acids. Variation of the ring-chain equilibrium constant among five amino acids correlated well with hydroxymethyl adduct equilibrium constants for model compounds. With histidine, a methylene bridge formed between the α-amine and an imidazole nitrogen, but this slowly gave way to a methylene bridge between the α-amine and C4 of the imidazole.

Attempts are being made to grow crystals of serum albumin large enough for x-ray crystallography.

. . . . . Dr. R. B. Simpson

VII. HORMONES AND RECEPTORS

Insulin Receptors in Genetic Disorders of Insulin Resistance

We have continued to study genetic disorders of peripheral resistance to the polypeptide hormones, insulin and somatomedin (insulin-like growth factor), using fibroblasts established in culture from skin biopsies from affected patients and control subjects. We have characterized specific cell surface receptors on fibroblasts for insulin and somatomedins, as well as the biological responses (glucose incorporation, amino acid transport) to these peptides. We have identified the first genetic defect of insulin receptors in fibroblasts cultured from a patient with leprechaunism, intrauterine growth retardation, and clinical insulin resistance. Insulin-stimulated glucose incorporation, mediated by the insulin receptor, is impaired in the patient's cells. We also have identified impaired binding for the polypeptide hormone most closely related to insulin, namely, insulin-like growth factor I (IGF-I) to specific IGF-I receptors, and a corresponding defect in IGF-stimulated glucose incorporation. These results suggest that insulin receptors and IGF receptors are structurally similar and may be similarly coupled to effector pathways. Further studies should help elucidate the relationship of hormone binding to the transmission of biological signals, and define the hormonal regulators of intrauterine growth.

. . . . . Dr. M. M. Rechler, and J. A. Romanus

Multiplication-Stimulating Activity (MSA): Rat Insulin-Like Growth Factor II

In our studies of the insulin-like growth factor (somatomedin) multiplication stimulating activity (MSA), a polypeptide synthesized by a cloned line of rat liver cells (BRL 3A), progress has been made in the following areas: (i) demonstration of a biosynthetic precursor of MSA (presumably pre-pro-MSA) in a cell-free translation system; (ii) demonstration by affinity crosslinking of two IGF receptor subtypes that differ in reactivity with insulin, IGF-I and IGF-II and in subunit structure; (iii) demonstration that insulin and MSA utilize insulin and IGF receptors, respectively, to mediate the same biological responses in hepatocytes, chondrocytes, myocytes, fibroblasts, and embryonal carcinoma cells; (iv) demonstration that MSA is the major somatomedin in fetal rat serum, and that MSA levels fall shortly after birth; (v) characterization of growth hormone-dependent binding proteins for somatomedins in human serum and fetal and postnatal rat serum.

. . . . . Dr. M. M. Rechler, J. A. Romanus, Dr. A. M. Acquaviva, Y. W.-H. Yang, Drs. M. A. DeVroede, N. Sasaki, C. Bruni (LBP, NIADDK); Drs. M. Kasuga, G. L. King and C. R. Kahn (Joslin Diabetes Center, Boston); Drs. S. P. Nissley, S. O. Adams, T. P. Foley and R. M. White (MD, NCI); Dr. W. B. Anderson (LPP, NCI): Dr. V. C. Hascall (LB, NIDR); Dr. R. S. Bar (Univ. Iowa); and Dr. R. E. Humbel (Univ. Zurich, Switzerland)

Thyrotropin Receptors, Thyroid Regulation, and Pathophysiology

The structure of the glycoprotein component of the thyrotropin (TSH) receptor has been defined, using monoclonal antibodies and TSH crosslinking procedures. The monoclonal stimulating antibodies mimicking autoimmune immunoglobulins in Graves' disease have been shown to be directed at the ganglioside component of the TSH receptor. A TSH-regulated ADP ribosylation system in thyroid members has been linked to the coupling mechanism between receptor, regulation of adenylate cyclase activity, and desensitization. A clonal line of cultured rat thyroid cells has been developed as a clinical assay to measure autoimmune-stimulating antibodies in patients with Graves' disease. A serum factor interacting with TSH, in the image of thyroid binding globulins, has been defined. The TSH- and Graves' antibody receptor-modulated effect on growth has been shown to be real and independent of cAMP regulatory paths.

. . . . . Drs. S. M. Aloj, J. L. Cohen, W. A. Valente, P. Laccetti, E. F. Grollman, P. Vitti, and L. D. Kohn (LBP, NIADDK); Dr. S. Shifrin (DCBD, NCI); Dr. L. Beguinot (University of Naples, Italy); and Drs. F. S. Ambesi-Impiombato, R. Toccafondi, and C. Rotella (University of Florence, Italy)

Tetanus Receptors and Mechanism of Action

Mechanisms of retrograde axonal transport have been characterized as have been tetanus interactions with ganglioside-deficient neural cells.

. . . . . Dr. L. D. Kohn (LBP, NIADDK) and Drs. M. C. Hardegree and W. H. Habig (DBP, BB, FDA)

Interferon Receptors and Action

Interferon effects on TSH-regulated thyroid cell iodide uptake have been demonstrated. Relations between TSH receptor expression and antiviral protection by interferon have been characterized in terms of their ability to bypass species specificity and involve the glycoprotein component of the TSH receptor.

. . . . . Drs. E. F. Grollman, S. J. Weiss, S. M. Aloj, and L. D. Kohn (LBP, NIADDK) and Dr. R. M. Friedman (LEP, NIADDK)

Graves' Disease

Human Graves' monoclonal antibodies have been obtained and characterized using a heterohybridoma approach. Human monoclonal Graves' stimulators have been characterized.

. . . . . Drs. W. A. Valente and L. D. Kohn (LBP, NIADDK)

Thyroglobulin Biosynthesis and Degradation

27S Thyroglobulin has been synthesized in vitro for the first time and a model proposed to account for its in vivo synthesis. The increasing importance of carbohydrate to thyroglobulin structure has been further defined.

. . . . . Drs. S. Shifrin (DCBD, NCI); Dr. L. D. Kohn (LBP, NIADDK); and
Drs. E. Consiglio and G. Salvatore (University of Naples, Italy)

Electrochemical Ion Gradients as a Mechanism of Cellular Message Transmission

Gangliosides have been implicated as receptor components for thyrotropin (TSH), toxins, and interferon. Monoclonal antibodies to gangliosides offers an experimental approach to examine the role of gangliosides in cellular recognition and the biological response to ligands. A subset of the monoclonal antibodies, prepared to the solubilized TSH receptor components that block or mimic some of the effects of TSH, has been found to interact with gangliosides. Monoclonal antibodies have independently been developed to specific gangliosides, and their effects on cell growth and interferon bioactivity have been determined.

Studies in this laboratory have been aimed at evaluating the hypothesis that a primary action of TSH, tetanus toxin, and interferon is an effect on the membrane that results in altered ion fluxes across the membrane. These studies continue to support this view and have further defined specific mechanisms by which tetanus toxin and interferon affect the electrochemical ion gradient. Recent studies have characterized iodide transport mechanisms in thyroid cells in culture, as well as described TSH-induced alterations in iodide flux.

. . . . . Drs. E. F. Grollman and S. J. Weiss

VIII. BLOOD COAGULATION, TRANSGLUTAMINASE, AND RELATED FACTORS

Crosslinking of Human and Lamprey Fibrins

An improved laser light scattering technique, correlating fibrin clot rigidity as a function of the crosslinking of the α and γ fibrin subunits in vitro, and in plasma revealed interesting results. In fresh human plasmas where coagulation was initiated by addition of Ca^{2+} ions, γ-subunits crosslinked rapidly and completely to dimers; little or no α crosslinking was detectable for periods exceeding 24 hours. (In in vitro human systems, thrombin-activated Ca^{2+}-dependent Factor XIII, will rapidly crosslink fibrin γ-subunits to γ-γ dimers, as well as completely crosslinking the α subunits to higher oligomers at a much slower rate.) Maximum clot rigidity develops in a very short period where only γ-γ dimers are apparent. Lamprey fibrin only crosslinks γ-subunits to γ-γ dimers in vitro and in plasma. The rigidity of crosslinked human clots in vitro, and in plasma have been measured.

Lamprey and Salmon Fibrinogens

Investigation of the molecular structure of the large $A\alpha$ subunit of lamprey fibrinogen by chemical modification followed by enzymatic degradation has proven evasive but challenging.

Action of human plasmin on a mixture of reduced and alkylated pure salmon fibrinogen subunits shows a rapid hydrolysis of the Aα subunits, but much slower for Bβ and γ subunits. Presently one may only speculate if this is due to structure, availability, or subunit size.

Rabbit Uterine Epithelial Cells

This cell line has been maintained in culture for 2 years. Factor XIII-like transglutaminases and the possibility that the cells excrete uteroglobulin are under study.

. . . . . Dr. J. A. Gladner

The Relationship between Structure and Function in the Polymerization of Fibrinogen

Bovine fibrinogen methylated with the trimethyl oxonium ion resulted in the polymerization of the fibrinogen. In 10 minutes the extent of modification was 18 carboxyl groups esterified with a 70% degree of polymerization. The methylated fibrinogen was totally polymerized in 30 minutes with approximately 100 carboxyl groups modified. The rate of aggregation was maximum in 5 to 10 minutes of reaction time with 12 carboxyl groups methylated. After 10 minutes the rate of aggregation decreased rapidly; however, some 80 carboxyl groups continued to be modified. The location of the critical groups methylated leading to the polymerization of the fibrinogen was evaluated using the enzyme thrombin. N-Terminal group analysis by the Edman method revealed that at least three carboxylic acid residues in the N-terminal segment of fibrinopeptide-A in the structure of fibrinogen were esterified. These results implicate the methylation of carboxylic acid groups in the structure of fibrinopeptide-A as generating the polymerization of the fibrinogen, and therefore serves as a model for the thrombin-induced enzymatic polymerization of fibrinogen.

The Ultrastructure of Methylated Fibrinogen

The esterification of the fibrin polymer through methylation disrupts the formation of periodic fibers in generating the fibrin network. It is, therefore, possible to formulate a mechanism of carboxyl group involvement in the highly ordered structural occurrence of periodicity in fibrin polymerization.

The Interaction of Reptilase with Citraconylated Fibrinogen

Reptilase releases fibrinopeptide-A at a faster rate from canine citraconylated fibrinogen than from the unmodified fibrinogen. The additional negative charges introduced into the fibrinogen by this modification result in a more favorable environment for the enzymatic action of reptilase.

. . . . . . Dr. Albert J. Osbahr

Transglutaminase and the Proliferating Cells in the Host

In the past years, several pieces of evidence indicated that proliferating tissues in the host, such as the granulation tissue in wound healing, the conceptus, cancer, require the presence of transglutaminase for proper growth. (Transglutaminase is an enzyme catalyzing the isopeptide bond formation between a glutamine residue and primary amine.)

This report briefly summarizes findings attempting to understand the role of transglutaminase in these biological phenomena.

We have found that transglutaminase inhibitors given to pregnant mice resulted in the abortion of the fetuses. It turned out that fetuses taken out of the uterus after the application of the inhibitors developed normally into the blastocyst stage in tissue culture, while if left in the uterus these would have been aborted. Since the "conceptus" can be considered as an "allotransplant," these experiments indicate that transglutaminase protects the alien tissue. Experiments have been carried out which indicate that tumor cells proliferate in the host because their "strangeness" is covered. Tumor cells taken out from mice which received transglutaminase inhibitors can be used to generate killer lymphocytes in syngenic animals. These killer lymphocytes are "haplotype"-specific for the cells used to generate them.

With such killer cells, it could be demonstrated that fibrinogen attached, with the use of transglutaminase, to the surface of tumor cells inhibits the killing process. Earlier experiments using malignant plasma cells of the mouse indicated that fibrinogen attached by transglutaminase to surface proteins covered transplantation antigens. This is now confirmed by this laboratory showing that β_2-microglobulin is a substrate of transglutaminase. Similar observations were made using human lung fibroblast (W1-38), its SV40 virus-transformed variant (W1-38 VA 13), and lung carcinoma of human origin which were grown in tissue culture. When radiolabeled β_2m was incorporated into HLA on the surface of the proliferating cells, a portion of β_2m was found, either in the cell membrane or among the shed materials, some of it transformed into higher molecular weight forms by transglutaminase of the cells.

The picture emerging from these studies is that tumor cells grow in the host because transglutaminase attaches fibrinogen or some other protein to the tumor cell surface to cover up their "strangeness" (antigenicity) from the cellular immune system. This implies that by inhibiting transglutaminase, the cover would be dropped and the immune system could eliminate the tumor cells. This appears to be the case since mice cured with our newly developed transglutaminase inhibitors do not take up the same tumor again.

. . . . . Dr. K. Laki

IX. MECHANISM OF HYPERTENSION IN HYPERTENSIVE RATS

This project is based on the concept that a cause of hypertension may be lowered permeability in the capillaries with age, requiring higher arterial pressure to maintain transvascular fluid movement (TVM). The method of measuring this fluid movement is based on swelling of the rodent tail under a slight vacuum. It has been shown by the author that this TVM decreases with age. This study will be conducted in SHR strain of rats (genetically hypertensive) and compared with non-hypertensive WKY rats as controls; measurements will start with weanlings and continue until adulthood.

. . . . . Dr. S. M. Rosenthal

X. MYCOBACTERIUM

In the present study, dendritic cells showed slow movement at the beginning. The movement became more rapid after cultivation, with the cells eventually detaching from the culture vessel to swim freely in the medium. The detached cells exhibited various configurations through rapid appearance and disappearance of needle-like, fan-like, or other bizarre-shaped projections. Single cells moved forward and turned about in various directions. Aggregates of various numbers of cells were formed by attaching tails to each other or to some cell debris. In aggregates, each cell moved in its own direction, simulating a group of insects or birds flying in various directions at the same time. Death of cells usually occurred suddenly during active movements. This phenomenon reminds one of the story of a Greek who ran 26 miles and 385 yards to tell of the victory over the Persians, and then dropped dead. Mitotic figures of dendritic cells were observed, indicating that these cells are capable of proliferation *in vitro*.

. . . . . Dr. Y. T. Chang

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AM 23,140-24 LBP

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Biochemistry of Sulfur-Containing Compounds

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Simon Black, Ph.D.	Biochemist	LBP NIADDK
	and Chief, Section on Biochemistry of Amino Acids		LBP NIADDK
OTHER:	Chien-Hua Niu, Ph.D.	Senior Staff Fellow	LBP NIADDK

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Biochemical Pharmacology

SECTION
Section on Biochemistry of Amino Acids

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 3.0	PROFESSIONAL: 2.0	OTHER: 1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Aminoacyl tRNA synthetase regulation. One form of the valyl tRNA synthetase of yeast can be dissociated with separation of a heat-stable, nondialyzable acidic substance that stimulates the enzyme's action. The heat-stable material has now been separated into at least four fractions, one of which has been purified to homogeneity. It is a peptide of about 18,000 molecular weight and appears to contain two residues of half cystine per mole.

Evolution. The capacity of nucleotide bases to form hydrogen bonds with peptides has been further investigated with the interaction of cytosine with peptides of serine and threonine the central point of interest. This interaction, previously demonstrated in organic solvents, has now been shown to occur also in water when suitable derivatives are used.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 23,230-32 LBP

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Chemotherapy of Mouse Leprosy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Yao Teh Chang, M.D.	Research Pharmacologist	LBP NIADDK

COOPERATING UNITS (if any)

Dr. S. H. Sun, Pulmonary Branch, National Heart, Lung, and Blood Institute, NIH

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Section on Pharmacology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.3	1.0	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In the present study, dendritic cells showed slow movement at the beginning. The movement became more rapid after cultivation, with the cells eventually detaching from the culture vessel to swim freely in the medium. The detached cells exhibited various configurations through rapid appearance and disappearance of needle-like, fan-like, or other bizarre-shaped projections. Single cells moved forward and turned about in various directions. Aggregates of various numbers of cells were formed by attaching tails to each other or to some cell debris. In aggregates, each cell moved in its own direction, simulating a group of insects or birds flying in various directions at the same time. Death of cells usually occurred suddenly during active movements. This phenomenon reminds one of the story of a Greek who ran 26 miles and 385 yards to tell of the victory over the Persians, and then dropped dead. Mitotic figures of dendritic cells were observed, indicating that these cells are capable of proliferation in vitro.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 23,330-04 LBP

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Aldoheptose Biosynthesis and Its Regulation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: William G. Coleman, Jr., Ph.D. Research Microbiologist LBP NIADDK

COOPERATING UNITS (if any)

None

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INSTITUTE AND LOCATION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.7	1.0	1.7

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A mutant of Escherichia coli K-12 carrying a single mutation, designated rfaD, which affects the synthesis of the aldoheptose, L-glycero-D-mannoheptose, has been isolated. Phenotypically this mutant is novobiocin-hypersensitive with altered lipopolysaccharide (LPS) core biosynthesis. It was observed that the LPS of the rfaD mutant contained the aldoheptose D-glycero-D-mannoheptose rather than the normal L-glycero-D-mannoheptose. It is postulated that L-glycero-D-mannoheptose is formed from D-glycero-D-mannoheptose via a racemization. A crude assay to measure the epimerase activity has been developed. The following facts have been established: (i) The rfaD gene product, ADP-L-glycero-D-mannoheptose-6-epimerase, is necessary for the conversion of ADP-D-glycero-D-mannoheptose to ADP-L-glycero-D-mannoheptose. (ii) The nucleotide ADP-D-glycero-D-mannoheptose accumulates in rfaD mutant strains. Two chimeric colicin E1 plasmids carrying the coding sequence for the rfaD wild type allele have been selected, subcloned, and shown to complement the rfaD phenotype. These clones also result in an amplification of ADP-L-glycero-D-mannoheptose-6-epimerase activity.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 23,580-19 LBP

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Gene Expression in the Rat and Other Organisms

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Anthony V. Furano, M.D.	Medical Officer (Research)	LBP NIADDK
OTHERS:	David A. Epstein, Ph.D.	Staff Fellow	LBP NIADDK
	Eva Csordas-Toth, Ph.D.	Visiting Fellow	LBP NIADDK
	Frank R. Witney, Ph.D.	Guest Worker	LBP NIADDK

COOPERATING UNITS (if any)

None

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INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 4.8	PROFESSIONAL: 4.0	OTHER: 0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

All metazoans examined to date contain repetitive DNA elements, and suggestions for their function include coordination of the expression of tissue-specific genes, promotion of chromosomal rearrangements such as duplications, inversions, and deletions, a role in speciation, or even no function except self-propagation as "selfish" DNA. We devised a semiquantitative method to analyze all the repeat DNA families of the rat and have identified a number of different classes: ancestral elements (i.e., those present in a number of rodent genera), elements specific to the genus Rattus, transcribed elements, nontranscribed elements, very high repeat elements, and divergent repeat elements. We are now examining the genomic organization of selected families corresponding to these classes and are constructing "chromosomes" in vitro containing one or more of these types of elements in an attempt to assess some of the functions that repeat elements serve in eukaryotic genomes.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 23,630-17 LBP

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Biology of Complex Carbohydrates

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Victor Ginsburg, Ph.D.	Research Chemist	LBP NIADDK
	and Chief, Section on Biochemistry		LBP NIADDK
OTHERS:	Manfred B. Brockhaus, M.D.	Guest Worker	LBP NIADDK
	Laura Chi Huang, Ph.D.	IPA Appointee	LBP NIADDK
	John L. Magnani, Ph.D.	Staff Fellow	LBP NIADDK

COOPERATING UNITS (if any)

None

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.6	3.5	2.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Tumor-specific glycolipid antigens of monoclonal antibodies will be isolated and characterized. The purified glycolipids will be used to isolate human antibody-producing cells of the desired specificity. The cells will be "immortalized" by EB virus transformation or hybridomas will be prepared from them in order to obtain monoclonal antibodies.

Oligosaccharides will be isolated from natural sources. Oligosaccharides will be coupled to macromolecules in order to make antibodies specific for certain sugar sequences. The antibodies will be used in radioimmune assays for the occurrence of these sugar sequences in the glycolipids and glycoproteins of carbohydrates of cell surfaces in development and regulation or as tumor-specific antigens.

Carbohydrates on cell membranes that are receptors for toxins, hormones, and antibodies will be isolated and characterized.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 23,730-22 LBP

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Factors Involved in Blood Coagulation and Related Fields

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Jules A. Gladner, Ph.D. Research Chemist LBP NIADDK

COOPERATING UNITS (if any)
Ralph J. Nossal, Ph.D., Biophysicist, PSL, DCRT; Soo Il Chung, Ph.D., Senior Investigator, LB, NIDR; Hayden G. Coon, Ph.D., Senior Investigator, LCB, NCI; Marc S. Lewis, Ph.D., Senior Investigator, BEIB, DRS;

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INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 2.5	PROFESSIONAL: 2.0	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Laser light scattering has been employed to study the rigidity of human and lamprey crosslinked fibrin clots, in vitro and under physiological conditions. We are correlating clot rigidity as a quantitative function of the crosslinking fibrin subunits. Investigations of the molecular structure of salmon and lamprey fibrinogens and fibrins, in particular the Aα-subunits are under study. SV40 transformed rabbit uterine epithelial cells have been maintained in culture for two years.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AM 23,830-03 LBP

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Electrochemical Ion Gradients as a Mechanism of Cellular Message Transmission

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Evelyn F. Grollman, M.D.	Medical Officer (Research)	LBP NIADDK
OTHER:	Donatella Tombaccini, Ph.D.	Visiting Fellow	LBP NIADDK
	Stuart J. Weiss, Ph.D.	Staff Fellow	LBP NIADDK

COOPERATING UNITS (if any)
R. M. Friedman (LEP, NIAMDD); W. H. Habig, BTB, DBP, BB, FDA); M. C. Hardegree (BTB, DBP, BB, FDA); H. R. Kaback (Roche Institute of Molecular Biology, Nutley, New Jersey)

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SECTION
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INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.4	2.3	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Gangliosides have been implicated as receptor components for thyrotropin (TSH), toxins, and interferon. Monoclonal antibodies to gangliosides offers an experimental approach to examine the role of gangliosides in cellular recognition and the biological response to ligands. A subset of the monoclonal antibodies, prepared to the solubilized TSH receptor components that block or mimic some of the effects of TSH, has been found to interact with gangliosides. Monoclonal antibodies have independently been developed to specific gangliosides, and their effects on cell growth and interferon bioactivity have been determined.

Studies in this laboratory have been aimed at evaluating the hypothesis that a primary action of TSH, tetanus toxin, and interferon is an effect on the membrane that results in altered ion fluxes across the membrane. These studies continue to support this view and have further defined specific mechanisms by which tetanus toxin and interferon affect the electrochemical ion gradient. Recent studies have characterized iodide transport mechanisms in thyroid cells in culture, as well as described TSH-induced alterations in iodide flux.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 23,960-16 LBP

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Cell Regulation by the Action of Pharmacodynamic Agents on the Cell Membrane

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Leonard D. Kohn, M.D.	Medical Director, USPHS	LBP NIADDK
	and Chief, Section on Biochemistry of Cell Regulation		LBP NIADDK
OTHERS:	Salvatore M. Aloj, M.D.	Visiting Scientist	LBP NIADDK
	Joshua L. Cohen, M.D.,Ph.D.	Medical Staff Fellow	LBP NIADDK
	Paolo Laccetti, Ph.D.	Guest Worker	LBP NIADDK
	George Lee, Ph.D.	Senior Staff Fellow	LBP NIADDK
	(temporarily assigned to Extramural Activities Program, NIADDK)		
	William A. Valente, M.D.	IPA Appointee	LBP NIADDK
	Paolo Vitti, M.D.	Visiting Fellow	LBP NIADDK

COOPERATING UNITS (if any) S. Shifrin (DCBD, NCI); E. F. Grollman (LBP, NIADDK); R. M. Friedman (LEP, NIADDK); W. H. Habig and M. C. Hardegree (BB, FDA); L. Beguinot, F. Beguinot, E. Consiglio, G. Salvatore (Univ. of Naples, Italy); C. Rotella, R. Toccafondi (Univ. of Florence, Italy)

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Laboratory of Biochemical Pharmacology

SECTION
Section on Biochemistry of Cell Regulation

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
7.1	6.0	1.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The structural and functional relationship in the mechanism by which glycoprotein hormones [thyrotropin (TSH), luteinizing hormone (LH), human chorionic gonadotropin (hCG), and follicle-stimulating hormone (FSH)], certain bacterial toxins (cholera and tetanus, for example), and the antiviral protective agent, interferon, interact with and transmit their message through the cell membrane is being further defined. Studies using monoclonal antibodies have continued which explore the importance of these relationships to the expression of thyroid hyperfunction in Graves' disease; to organ-specific autoimmunity in general and the autoimmunity of Graves' disease in particular; to fluid losses in intestinal diarrhetic states; to thyroid storm and the sympathetic overactivity syndrome of tetanus; to the ability of hormones to modulate the oncogenic state; and to the mechanism by which toxins subvert normal mechanisms to impose their pathological effects. Studies have been continued which evaluate the role of membranes in thyroglobulin biosynthesis and thyroglobulin biodegradation to T3 and T4 and the role of carbohydrate moieties in thyroglobulin structure and post-translational processing. Studies also continue to explore lipid regulation of receptor expression with special emphasis on neuronal and thyroid cell development.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 23,970-07 LBP

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)
Cancerostatic and Enzyme Regulating Agents and Intermolecular Interactions

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Koloman Laki, Ph.D.	Research Chemist	LBP NIADDK
	and Chief, Section on Physical Biochemistry		LBP NIADDK
OTHERS:	Zsuzsanna Hernadi, Ph.D.	Visiting Fellow	LBP NIADDK
	Pamela J. Baker, Ph.D.	Guest Worker	LBP NIADDK
	Thomas G. Burke, M.S.	Guest Worker	LBP NIADDK
	James E. Girard, Ph.D.	Guest Worker	LBP NIADDK
	Elizabeth Rabold, M.S.	Guest Worker	LBP NIADDK

COOPERATING UNITS (if any)
Dr. A. Mukherjee (PRB,NICHHD); Dr. S. T. Yancey (LCHPH,NCI); Dr. L. Fesus, Departments of Internal Medicine and Central Clinical Chemistry, Medical University, Debrecen, Hungary.

LAB/BRANCH
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SECTION
Section on Physical Biochemistry

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 3.0	PROFESSIONAL: 2.5	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We find that inactivation of transglutaminase with neopentyl isocyanate generated from nitrosourea in tumor-bearing or pregnant mice results in the appearance of surface antigens which stimulated T-cells and these kill unmasked tumor cells and fetal cells. Our explanation is that inactive transglutaminase leaves the tumor's "altered self" intact; thus the "strangeness" of the tumor cell is free to stimulate the cellular immune system for the killing. Factor XIII (transglutaminase)-deficient women not able to "mask" the fetus, abort it. With the addition of Factor XIII, the pregnancy is successful. This finding suggests that what we see with tumor-bearing syngenic mice might be applicable to autochtonous tumors as well.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 24,020-19 LBP

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Cell Surfaces: (a) Phagocytosis and Adhesion in Macrophages (b) E. coli

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Loretta Leive, Ph.D.	Research Biologist	LBP NIADDK
OTHERS:	Robert C. Goldman, Ph.D.	Staff Fellow	LBP NIADDK
	Nili Grossman, Ph.D.	Visiting Fellow	LBP NIADDK
	Seiichi Takasaki, Ph.D.	Visiting Fellow	LBP NIADDK
	Yoshiko Takasaki, M.A.	Guest Worker	LBP NIADDK

COOPERATING UNITS (if any)

Dr. John D. Foulds, Laboratory of Biochemistry and Metabolism, NIADDK, NIH

LAB/BRANCH
Laboratory of Biochemical Pharmacology

SECTION
Section on Pharmacology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 4.3	PROFESSIONAL: 3.0	OTHER: 1.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In studies with macrophage cell lines, we have been able to induce Fc-mediated phagocytosis in a line lacking it by short exposure to DMSO. We have also selected and characterized mutants that are reduced in generalized phagocytosis.

We have studied Salmonellae that differ in pathogenicity for mice solely because of a difference in lipopolysaccharide (LPS) side chains, and found that the different polysaccharides differ in degree of activation of complement via the alternative pathway in an antibody-independent fashion. The degree of activation determines the subsequent ingestion of the bacteria by macrophages, presumably via the Fc receptor. It is likely that this mechanism of pathogenicity is operative in vivo.

LPS is heterogeneous in side chain length, which varies from 0 to more than 40 repeating units per molecule. We find heterogeneity is maintained in mutants almost completely blocked in ability to make side chain, indicating preference of biosynthetic enzymes for certain rather long lengths. We have also demonstrated and characterized a long polysaccharide with the composition of LPS side chain but notattached to lipid A, loosely attached to the cell surface, that probably accounts for certain false capsule responses.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 24,120-11 LBP

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Mechanism of Hypertension in Rats
(formerly "Studies in Traumatic Shock and Cellular Immunity")

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Sanford M. Rosenthal, M.D.	Scientist Emeritus	LBP NIADDK

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Biochemical Pharmacology

SECTION
Section on Pharmacology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 1.2	PROFESSIONAL: 1.0	OTHER: 0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is based on the concept that a cause of hypertension may be lowered permeability in the capillaries, with age, requiring higher arterial pressure to maintain transvascular fluid movement (TVM). The method of measuring this fluid movement is based on swelling of the rodent tail under a slight vacuum. It has been shown by the author that this TVM decreases with age. This study will be conducted in SHR strain of rats (genetically hypertensive) and compared with non-hypertensive WKY rats as controls; measurements will start with weanlings and continue until adulthood.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 24,140-16 LBP

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)
Tryptophan Synthase: Structure, Function, and Subunit Interaction
(formerly "Chemistry and Mechanism of Pyridoxal Phosphate Enzymes")

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Edith W. Miles, Ph.D.	Research Chemist	LBP NIADDK
OTHERS:	Katsuyuki Tanizawa, Ph.D.	Visiting Fellow	LBP NIADDK
	Katsuhida Yutani, Ph.D.	Visiting Scientist	LBP NIADDK

COOPERATING UNITS (if any)
Drs. K. Yutani and K. Ogasahara, Institute for Protein Research, Osaka University, Osaka, Japan; Drs. D. Houck and H. G. Floss, Department of Medicinal Chemistry and Pharmacognosy, Purdue University, West Lafayette, Indiana; Dr. Thomas Fairwell, Molecular Disease Branch, NHLBI, NIH

LAB/BRANCH
Laboratory of Biochemical Pharmacology

SECTION
Section on Pharmacology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.6	2.4	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Tryptophan synthase. The structure, function, and subunit interaction in the multienzyme complex are being investigated. The alpha subunit contains 2 independently folding domains which can be separated after limited proteolysis. Circular dichroism studies show that the guanidine hydrochloride-induced unfolding of the alpha subunit is stepwise: the smaller C-terminal domain unfolds at low concentrations of denaturant to yield a partially unfolded intermediate; the larger N-terminal domain unfolds at higher concentrations of denaturant. The sites of interaction between the alpha and beta subunits and between the alpha domains are being mapped by chemical modification experiments. An active site arginine in the beta subunit has been modified by phenylglyoxal and is being located in the sequence. The stereochemistry of the sodium borohydride reduction of Schiff bases of the coenzyme, pyridoxal phosphate, with amino groups of a substrate and of an active site lysine, has been determined.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AM 24,150-11 LBP

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Intermolecular Interactions in Physical Biochemistry

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Allen P. Minton, Ph.D.	Research Chemist	LBP NIADDK
OTHERS:	Jacob Wilf, Ph.D.	Visiting Fellow	LBP NIADDK
	Paul Karmin	Biological Aid	LBP NIADDK

COOPERATING UNITS (if any)
Kenneth W. Minton, M.D., Depts. of Radiology and Pathology, Stanford University Medical Center.

LAB/BRANCH
Laboratory of Biochemical Pharmacology

SECTION
Section on Pharmacology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 2.4	PROFESSIONAL: 2.2	OTHER: 0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A new kinetic model for the formation of fibrin gel has been proposed which invokes the formation of a soluble complex of fibrinogen and fibrin as an intermediate. Chromatographic studies of fibrinogen/fibrin solutions at various degrees of progress toward gelation reveal the formation of a soluble species of the appropriate molecular weight. The rate and/or extent of fibrin gelation has been found to be strongly affected by the type and amount of added "inert" macromolecules. The ability of proteins to regain native structure and/or enzymatic activity after exposure to elevated heat or high ethanol concentrations has been found to be strongly dependent upon the type and amount of added "inert" macromolecules.

Key words: fibrin gelation, gel chromatography, kinetic model, protein stability.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 24,260-16 LBP

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)
Enzymatic Mechanisms of DNA Replication: The Bacteriophage T4 System
(formerly "Enzymatic Studies of Nucleic Acid Metabolism")

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Nancy G. Nossal, Ph.D.	Research Chemist	LBP NIADDK
OTHERS:	Lynn L. Silver, Ph.D.	Staff Fellow	LBP NIADDK
	Malabi Venkatesan, Ph.D.	Visiting Fellow	LBP NIADDK

COOPERATING UNITS (if any)
Drs. K. Williams, E. Spicer, and W. Konigsberg, Yale University, New Haven, Connecticut

LAB/BRANCH
Laboratory of Biochemical Pharmacology

SECTION
Section on Pharmacology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.3	2.0	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)
We are studying the reactions required for the replication of duplex DNA *in vitro* using proteins encoded by T4 bacteriophage. The T4 gene 41 and T4 gene 61 priming proteins synthesize ribopentanucleotides of sequence pppACNNN which are elongated by the T4 gene 43 DNA polymerase, T4 gene 45 protein, the complex of the T4 gene 44 and T4 gene 62 proteins (a DNA-dependent ATPase), and the T4 gene 32 helix destabilizing protein. The stoichiometry of the priming reaction suggests that it is catalyzed by a complex of an oligomer of 41 protein and a 61 protein monomer. The T4 41 protein is also a DNA helicase which unwinds short fragments annealed to single-stranded DNA in a 3' to 5' direction, and increases the rate of chain elongation during strand displacement synthesis on nicked duplex DNA by T4 DNA polymerase and the T4 44/62, 45, and 32 proteins. The gene 44/62 and 45 polymerase accessory proteins permit the polymerase to bind more tightly to DNA in an ATP-dependent reaction. They thus facilitate the movement of the polymerase through kinetic barriers in the DNA in both the 5' to 3' polymerization and 3' to 5' exonuclease reactions. We are searching for T4 or host proteins required for the initiation of replication of duplex T4 DNA.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 24,360-08 LBP

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Action of Thrombin on Fibrinogen and Study of Related Blood Clotting Mechanisms

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Albert J. Osbahr, Ph.D.	Research Biochemist	LBP NIADDK

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Biochemical Pharmacology

SECTION
Section on Physical Biochemistry

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	1.0	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Bovine fibrinogen methylated with the trimethyl oxonium ion resulted in the polymerization of the fibrinogen. In 10 min the extent of modification was 18 carboxyl groups esterified with a 70% degree of polymerization. The methylated fibrinogen was totally polymerized in 30 min with approximately 100 carboxyl groups modified. The rate of aggregation was maximum in 5 to 10 min of reaction time with 12 carboxyl groups methylated. After 10 min the rate of aggregation decreased rapidly; however, some 80 carboxyl groups continued to be modified. The location of the critical groups methylated leading to the polymerization of the fibrinogen was evaluated and a minimum of three carboxylic acid residues were esterified in the N-terminal segment of fibrinopeptide-A in the structure of fibrinogen. These results implicate the methylation of carboxylic acid groups in the structure of fibrinopeptide-A as generating the polymerization. The esterification of fibrin polymer through methylation disrupts the appearance of periodic fibers in the formation of the fibrin network. Reptilase releases fibrinopeptide-A at a faster rate from canine citraconylated fibrinogen than from the native fibrinogen.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AM 24,390-09 LBP

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)
Hormones and Receptors: Insulin and Insulin-Like Growth Factors (Somatomedins)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Matthew M. Rechler, M.D.	Medical Director, USPHS	LBP NIADDK
OTHERS:	Angela M. Acquaviva, Ph.D.	Guest Worker	LBP NIADDK
	Monique A. DeVroede, M.D.	Guest Worker	LBP NIADDK
	Yvonne W.-H. Yang	Guest Worker	LBP NIADDK
	Norio Sasaki, M.D.	Visiting Associate	LBP, NIADDK
	Carmelo Bruni, M.D.	Visiting Scientist	LBP, NIADDK

COOPERATING UNITS (if any) S. P. Nissley, S. O. Adams, T. P. Foley and R. M. White (MB, NCI); W. B. Anderson (LPP, NCI); M. Kasuga, G. L. King and C. R. Kahn (Joslin Diabetes Center, Boston); R. E. Humbel (Univ. Zurich, Switzerland); R. S. Bar (Univ. Iowa); V. C. Hascall (LB, NIDR)

LAB/BRANCH
Laboratory of Biochemical Pharmacology

SECTION
Section on Biochemistry of Cell Regulation

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6.0	4.5	1.5

CHECK APPROPRIATE BOX(ES)
☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Fibroblasts cultured from a patient with the syndrome of leprechaunism (characterized by physical abnormalities, intrauterine growth retardation, and clinical insulin resistance) exhibit abnormalities of insulin receptors, receptors for insulin-like growth factors (IGFs), and impaired stimulation of glucose incorporation by these hormones through these receptors. In our studies of the insulin-like growth factor (somatomedin) multiplication stimulating activity (MSA), a polypeptide synthesized by a cloned line of rat liver cells (BRL 3A), progress has been made in the following areas: (i) demonstration of a biosynthetic precursor of MSA (presumably pre-pro-MSA) in a cell-free translation system; (ii) demonstration by affinity crosslinking of two IGF receptor subtypes that differ in reactivity with insulin, IGF-I and IGF-II and in subunit structure. (iii) demonstration that insulin and MSA utilize insulin and IGF receptors, respectively, to mediate the same biological responses in hepatocytes, chondrocytes, myocytes, fibroblasts and embryonal carcinoma cells; (iv) demonstration that MSA is the major somatomedin in fetal rat serum, and that MSA levels fall shortly after birth; (v) characterization of growth hormone-dependent binding proteins for somatomedins in human serum and fetal and postnatal rat serum.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 24,590-11 LBP

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Structure and Interactions of Biologically Important Macromolecules

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Harry A. Saroff, Ph.D.	Scientist Emeritus	LBP NIADDK

COOPERATING UNITS (if any)
Clinical Endocrinology Branch, NIADDK, NIH

LAB/BRANCH
Laboratory of Biochemical Pharmacology

SECTION
Section on Pharmacology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	1.0	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Cooperative binding systems involving aggregation, redistribution, subunit interactions, and ligand interactions are being formulated and studied in hemoglobin and other proteins related to hormone action.

Slow degradation of hemoglobin, collagen, immunoglobulins, and albumin is under investigation in order to define and explain the phenomenon.

Monte Carlo analyses are done to yield probabilities for uniqueness and homology of amino acid sequences.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AM 24,640-11 LBP

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

(I) Reactions of Formaldehyde; (II) Crystallization of Serum Albumin [formerly "(I) Oxidation of Hemoglobin and (II) Reactions of Formaldehyde"]

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Richard B. Simpson, Ph.D.	Research Chemist	LBP NIADDK

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Biochemical Pharmacology

SECTION
Section on Pharmacology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	1.0	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The reactions of formaldehyde with amino acids and diamines were studied. The rates and, where possible, the equilibria for methylene bridge ring formation were determined. The slow step was one of three possibilities: (1) intermolecular reaction of formaldehyde with amino acid; (2) removal of hydroxyl from the product of this reaction; or (3) ring closure. The rate of the ring closure step varies by more than a billion from phenylalanine to some of the other amino acids. Variation of the ring-chain equilibrium constant among five amino acids correlated well with hydroxymethyl adduct equilibrium constants for model compounds. With histidine, a methylene bridge formed between the α-amine and an imidazole nitrogen, but this slowly gave way to a methylene bridge between the α-amine and C4 of the imidazole.

Attempts are being made to grow crystals of serum albumin large enough for x-ray crystallography.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 24,709-01 LBP

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)
Purification of Ornithine Decarboxylase and Regulation of S. cerevisiae

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Celia White Tabor, M.D. Medical Director, USPHS LBP NIADDK

COOPERATING UNITS (if any)
Drs. H. Tabor and A. K. Tyagi (LBP, NIADDK)

LAB/BRANCH
Laboratory of Biochemical Pharmacology

SECTION
Section on Biochemistry of Amino Acids

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.8	1.0	0.8

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)
We have continued our studies on the purification and regulation of ornithine decarboxylase of S. cerevisiae, the rate-limiting enzyme in the biosynthesis of putrescine, spermidine, and spermine. We have prepared an antibody column, which permits us to obtain 70% pure enzyme in one step. We have also developed immunological methods that permit us to study an enzyme in a crude fresh extract. We have now found evidence that the enzyme in vivo differs in size (80,000 MW) from the enzyme which we purified by standard techniques (68,000 MW). The changes result from proteolysis, since addition of a proteolytic inhibitor markedly slows the conversion. Strains with high enzyme activity, our mutants (spe10) lacking ornithine decarboxylase, cells which have lost their ornithine decarboxylase activity as a result of growth with amines, and wild type cells with very low activity, all have equal enzyme protein bands in the 86,000 position by our techniques. Thus post-translational modification must account for changes in enzyme activity.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AM 24,710-32 LBP

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)
Estimation, Metabolism, and Function of Amines

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Herbert Tabor, M.D.	Medical Director, USPHS	LBP NIADDK
	and Chief, Laboratory of Biochemical Pharmacology,		LBP NIADDK
	and Chief, Section on Pharmacology		LBP NIADDK
OTHER:	Anil K. Tyagi, Ph.D.	Visiting Associate	LBP NIADDK

COOPERATING UNITS (if any)

Dr. C. W. Tabor (LBP, NIADDK)

LAB/BRANCH
Laboratory of Biochemical Pharmacology

SECTION
Section on Pharmacology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 4.0	PROFESSIONAL: 2.0	OTHER: 2.0

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In previous studies we have isolated the various enzymes concerned with polyamine biosynthesis in Escherichia coli and have defined and mapped the genes concerned with the biosynthesis of these enzymes. We have constructed mutants deficient in each of these steps and have prepared strains that cannot synthesize polyamines. The purpose of these studies is to find what the action of polyamines is in vivo, i.e., the physiological role of the polyamines.

In our more recent studies we have found that these mutants are defective in the translation step in protein biosynthesis in vivo. We have found that changes in the S12 ribosomal protein resulting from a rpsL (strA) mutation cause a slow-growing polyamine-deficient strain to cease growing; i.e., we now have an absolute polyamine-requiring auxotroph of E. coli. We have also shown a marked defect in the multiplication of amber mutants of bacteriophage T4 and T7 in polyamine-deficient strains and accumulation of an amber fragment. These results indicate that polyamines are involved in the stability or conformation of the ribosomal complex involved in protein biosynthesis in vivo.

We have also constructed a plasmid containing the gene for adenosylmethionine decarboxylase that is suitable for DNA sequencing.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 24,940-09 LBP

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

The Killer Double-Stranded RNA Plasmids of *Saccharomyces cerevisiae*

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Reed B. Wickner, M.D.	Senior Surgeon, USPHS	LBP NIADDK
OTHERS:	Steven G. Ball	Guest Worker	LBP NIADDK
	Susan Porter Ridley, Ph.D.	Staff Fellow	LBP NIADDK
	Steve S. Sommer, M.D., Ph.D.	Medical Staff Fellow	LBP NIADDK
	Micheline Wesolowski	Guest Worker	LBP NIADDK

COOPERATING UNITS (if any)

Faculte des Sciences Agronomiques de l'Etat, B5800, Gembloux, Belgium

LAB/BRANCH
Laboratory of Biochemical Pharmacology

SECTION
Section on Pharmacology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 4.3	PROFESSIONAL: 4.1	OTHER: 0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The presence of double-stranded RNAs M1 or M2 in virus-like particles enables cells to secrete the K1 or K2 toxins and be immune to these toxins. We have defined 29 chromosomal genes (called makl, . .) needed to replicate the M dsRNA and 5 regulatory chromosomal genes affecting this process (ski genes, KRB1). M2 is excluded by M1 dsRNA. Another plasmid, called [EXL], also excludes M2. Yet another, [NEX], prevents this effect of [EXL] on M2. A third, [HOK], acts as a helper for a replication-defective mutant of M1. Three very different L dsRNAs (L-A, L-B, and L-C], all the same size (3 x 10 to the 6th daltons), are present in various strains. [EXL], [NEX], and [HOK] are located on variants of L-A. L-B or L-C are stably maintained in the same cell as L-A. Virus-like particles in strains carrying only L-B or L-C have different proteins than those carrying L-A.

ANNUAL REPORT OF THE
LABORATORY OF CHEMICAL BIOLOGY

The Laboratory of Chemical Biology conducts research on fundamental problems of cell biology, relating the chemistry of macromolecules to cell function; on the structure, function and dynamics of macromolecules; and on genetics and developmental biology. During the last year an increasing fraction of the Laboratory's efforts have dealt with new programs in cellular, molecular and developmental biology, as well as a new study of protein conformational dynamics. These projects include biophysical, biochemical, immunological and molecular genetic approaches to biological questions. In addition, the several projects in peptide synthesis and peptide-protein interactions have moved in novel directions. Lastly, the program on sickle cell anemia is now involved in several clinical projects.

Among the major new areas of exploration in the Laboratory of Chemical Biology are the use of an erythroleukemic cell line as both a model for studying the control of globin gene expression and as an assay system for studying the expression of genes transfected into these cells; the use of monoclonal antibodies and protein biosynthetic labels and inhibitors to study gene expression in the maturing oocyte and embryo; the isolation, characterization and use of yeast cytochrome c synthetase in order to synthesize an ancestral cytochrome c; and the development of new methodology for using enzymes and labile blocking groups to synthesize peptide bonds. The program on the molecular pathogenesis of sickle cell anemia has moved into analyses of the pathophysiological basis of the other sickle cell syndromes, especially hemoglobin SC disease; the project on the development of non-covalent inhibitors of sickle hemoglobin gelation has progressed to an analysis, by difference Fourier X-ray crystallography, of the binding of several gelation inhibitors to hemoglobin crystals; and the entire program is concentrating on new methods of evaluating disease severity and response to therapy in patients.

Section on Protein Chemistry

The three major proteins which are being studied in this Section are human leukocyte interferon, bovine neurophysin and paramecium cell surface antigens. The project on interferon is centered around the chemical synthesis, by solution and solid phase methods, of peptides from the carboxy-terminal end of the protein and the investigation of biological and immunological function in these peptides. The goal of the work is to identify regions of the protein with specific functions, as this information may facilitate the development of interferon analogues which have certain of their medical uses improved. As part of this work, a major effort has been devoted to improvement of the general techniques for chemical synthesis of peptides. In particular, the conditions for the use of trypsin, in the presence of methylamine, to form peptide bonds have been defined. In addition, two new protecting groups, citraconyl and phenylacetyl, have been introduced for solid phase chemical synthesis and these may have widespread utility.

The program on peptide-protein interactions in neurophysin has concentrated on the study of the binding and self-association of this protein to affinity columns of a tripeptide. The effects on binding and self-association and on the antigenicity of the protein, of several chemical modifications have been studied. In addition, a tumor line which secretes neurophysin is being sought to allow biosynthetic studies of neurophysin and its related hormones. Chemical synthesis of parts of neurophysin (semisynthesis) is being initiated after a period of development of this methodology with ribonuclease semisynthesis.

The paramecium cell surface antigens, which are responsible for the mating reaction, have been studied by light and fluorescence microscopy as well as by electron microscopy, using fluorescent and ferritin probes. Cellular localization, surface movements, and the effects of antibodies on these proteins have been examined. The binding of certain paramecium strains to polystyrene has been investigated as a model of the mating reaction. In a collaborative effort, protein chemical techniques have been used to help localize the peptides in the λ CRO repressor protein that bind to DNA.

Section on Protein Conformation

During the last year the efforts of this Section's work on protein folding and dynamics have centered on the study of cytochrome *c*. One project has as its goal the synthesis of cytochrome *c* by chemical and enzymatic means with the particular aim of synthesizing the ancestral cytochrome *c* of eukaryotes to learn of protein structure-function factors involved in the evolution of this gene which is central to all cell function. To this end chemical synthesis of three large fragments of the protein is being done, as is the isolation and characterization of the cytochrome *c* synthetase which forms the thioether bond to the heme. The enzyme has been solubilized and partially purified and has been used to attach the heme moiety to a synthetic fragment corresponding to residues 1-25. Chemical synthesis of other fragments is underway.

Another project has been the continued characterization of a three fragment non-covalent complex (with the heme attached to one fragment) of the protein. Several analogues of one of the fragments have been chemically synthesized and the dissociation constants of these complexes determined. The results indicate that modulation of the global cooperative interactions occur, without large conformational changes, between different energy states and are related to the mechanism of increasing the oxidation-reduction potential. A third project, on kinetic and equilibrium measurements of two fragment complementing systems of cytochrome *c*, has led to results which indicate that the global cooperative interactions that stabilize the three-dimensional structure occur late in folding, not early as many kinetic models of folding assume.

Section on Macromolecular Biology

The efforts of this Section are concentrated into two major areas. One group is interested in hemoglobin and erythroid cell biology, at both a basic and a medical level; a second group is studying gene expression in maturing mouse oocytes and embryos. The work on hemoglobin has centered on experimental and theoretical approaches to understanding the intracellular polymerization of sickle hemoglobin in sickle cell anemia and other sickle-syndromes, especially hemoglobin SC disease. New methods of nuclear magnetic resonance spectroscopy, cell fractionation, and electron microscopy have been developed to study the intracellular polymerization and new theoretical analyses involving protein and water non-ideality have been developed to explain the results and to make predictions about *in vivo* behavior. Several pilot clinical studies have been initiated, which are designed to predict the severity of this disease and the response to therapy. The work on gelation inhibitors has continued through the synthesis and assay of new sets of compounds which prevent the intermolecular assembly process, as well as through a collaborative study with the Medical Research Council Laboratory of Molecular Biology in Cambridge, England to determine the site of binding, and thus the mode of action at the atomic level, of several of the peptide inhibitors of sickle hemoglobin gelation. A study of the gelation process as a function of osmotic stress has led to new insights into the thermodynamics of the phase transition and how polymerization might affect the water of the sickle erythrocyte.

The erythroid cell biology studies have largely concentrated on the K562 human erythroleukemic cell line. It has been shown that hemin affects, in a reversible manner, the accumulation of various hemoglobin species in these cells. Recent results now show that this depends upon both changes in the transcription of the specific globin genes and in their translation into protein and that the effects on the gene clusters is not fully coordinate. In addition, the K562 cells are being used to develop a human cell "assay system" for the expression of the human globin genes. These cells have been transfected with a plasmid which contains the β-globin gene and the messenger RNA synthesized is being analyzed by molecular hybridization with the appropriate cDNA. A "library" of the K562 DNA is also being constructed. These studies should clarify the control of globin gene synthesis in these cells and, possibly, all human erythroid cells.

A related project is the study of hemoglobin degradation in erythroid cells (the K562 line) and in erythrocytes themselves. Recently, this work has led to the identification of an enzyme in the human malarial parasite, P. falciparum, which appears to be specific for hemoglobin degradation and may allow a new approach to malaria therapy.

The other program in this Section is concerned with gene regulation in early mammalian development. The biosynthesis of the three mouse zona pellucida glycoproteins has been studied and it has been shown that tunicamycin inhibits their secretion. The synthesis of these proteins stops upon oocyte maturation. Conversely, it has been found that proteoglycan synthesis occurs only in the early embryo. Recently monoclonal antibodies have been developed to these proteins and these antibodies are being used to isolate specific messenger RNAs, and with fluorescent labels, to localize the proteins on oocytes. In a related project, endonucleases have been used to develop an assay for decondensation of sperm nuclei after reduction and alkylation. This assay is being used to seek protein(s) in the mouse oocyte that may be involved in this important step in the processing of sperm nuclei after fertilization.

SECTION ON PROTEIN CHEMISTRY

I. Human Leukocyte Interferon

A. Localization of Biological Function

For elucidation of structure and function relationships in human leukocyte interferon αA and αD, a number of peptides from the C-terminal region were synthesized by conventional solution synthesis and solid phase synthesis and their biological and immunological characteristics were investigated. The peptide 121-166 was shown to contain antigenic determinants and used to produce antibodies specific for native leukocyte interferon. Analysis using smaller peptides resulted in localization of two distinct determinants in this sequence. Assays of antiviral activity and cellular membrane receptor binding are in progress. (Smith, Ohno, Widmer, Nelson)

B. Improvements in Peptide Synthesis

Attempts to develop further the methodology of enzymic peptide synthesis showed that trypsin - in the presence of methylamine - catalysed formation of peptide bonds of nonspecific amino acid residues such as glycine, alanine, serine, valine and histidine. Further, we have introduced two novel α-amino protecting groups compatible with enzymic coupling in aqueous phase: the acid labile citraconyl group and the phenylacetyl group which can be cleaved enzymatically with penicillinacylase. These new techniques will eventually be applied to the synthesis of other highly pure peptides of biological interest. (Widmer, Ohno)

II. Bovine Neurophysin

A. Quantitative Affinity Chromatography

This project is part of a continuing program to develop the use of quantitative affinity chromatography for the characterization of macromolecular interactions. As part of the current studies, adsorbents containing immobilized neuropeptide hormones and their analogues are being used to elucidate the binding interactions and ligand-induced self-association of bovine neurophysins (NP's). The quantitative chromatographic behavior observed with native NPII, and NPII, mononitrated at Tyr 49 to eliminate monomer bivalency, were compared. The chromatographic approach has been used to study the dependence of hormone-mediated NP self-association on the intactness of the hormone binding surface. This method also is being used to study the nature of interactions of active site peptide ligands with polypeptide fragment complexes of the thermostable protease, thermolysin. (Chaiken, Abercrombie, Dalzoppo)

B. Chemical Modifications

We have been investigating the structural requirements in neurophysins (NP's) for hormone binding, self-association, and antibody recognition. The principal approach during this period has been to determine the alteration in these properties in NP's that have been derivatized. Bovine NP derivatized by trypsin digestion and photo-affinity labelling of the hormone binding site were analyzed by affinity chromatography and radioimmunoassay. These data were correlated with radioimmunoassay data for disulfide-broken NP's and native NP's with different sequences. Overall, the results showed that NP hormone binding and self-association are critically dependent upon a structurally intact protein molecule. In contrast, antibody recognition is lost only in NP derivatives in which disulfide bonds are broken. (Chaiken, Abercrombie, Sequeira)

C. Biosynthesis

The project is a part of a general program investigating biochemical aspects of biosynthesis of neurophysins and neurophyophysial hormones, with a current emphasis on ectopic hormone-protein synthesis by tumors. The human tumor cell lines of small cell carcinoma of lung (SCCL) is a good model to study the relationship between tumor biology and ectopic peptide/protein biosynthesis. An attempt has been made to grow SCCL in vitro and screen them for the presence of neurohypophysial peptide hormones and neurophysins. Special attention will be focussed to elucidate the biochemical and immunological nature of vasopressin-neurophysin precursor(s). The understanding of ectopic hormone and protein biosynthesis by tumors may shed light on the nature of cellular differentiation and the process of malignant changes. Such a study also raises the possiblity of presymptomatic detection of small cell carcinoma of lung and the precise assessment of therapy. (Chaiken, Sequeira)

D. Semisynthesis

This project is part of the continuing studies, using protein semisynthesis, to understand the relationship between primary structure of proteins and the higher order properties of folding, interactions, and ultimate biological functions. Sequence modeling studies of ribonuclease have been undertaken, which involve the design and the chemical semisynthesis of model proteins with specific chemical and conformational properties directed artificially. The studies of semisynthetic model proteins have provided the insight into the mode of non-covalent interaction between RNase-(1-20) and RNase-(21-124). The studies of neurophysins and their precursors is also progressing through their semisynthesis in order to investigate hormone-protein interaction, folding of proteins, and biosynthetic pathway of hormones. (Chaiken, Kanmera)

III. Paramecium and Virus Proteins

A. Surface Molecules in Paramecia

When paramecia are placed in homologous immune sera they become immobilized and eventually die. This specific reaction has been shown to be due to a series of protein antigens associated with the cell surface. Fluorescence studies reveal a pattern of Ag-Ab redistribution over the cell surface analogous to the capping phenomenon reported in other cell types. The process of redistribution occurs at the tips of the cilia and frequently results in the agglutination of several cilia into groups. TEM studies on sections through the ciliary bundles of immobilized cells show the axonemes of these agglutinated cilia contained within a single unit membrane. This fusion of ciliary membranes is readily induced by intact immune IgG, but is not induced by Fab fragments prepared from the same IgG. Cross-linking of the Fab fragments with goat anti-rabbit IgG results in immobilization and membrane fusion identical to that seen with intact immune IgG. (Steers, Kitamura, Lee)

B. Attachment of Paramecia to Polystyrene

Mating reactive cells of Paramecium caudatum attach to the polystyrene surface of certain Petri dishes. This attachment occurs at the tips of ventrally located cilia (mating reacting cells) and appears to involve a hydrophobic interaction. Although the attachment is correlated with mating reactivity, the attachment sites are distinct from the mating reactive sites. Attachment of cells to polystyrene also triggers the early migration of the gametic nucleus which precedes meiosis during the normal conjugation process. Certain aromatic compounds have been shown to greatly enhance the attachment of cells without affecting their mating reactivity. (Steers, Kitamura, Lee)

C. Lambda CRO and DNA Binding

The λ CRO repressor protein has been modified by reductive methylation blocking the lysine residues to tryptic hydrolysis. Tryptic hydrolysis (restricted to arginyl residues) has resulted in four tryptides which are readily separated by HPLC. Double labelling experiments in which the methylation is carried out with either ^3H or ^{14}C formaldehyde in the presence and absence of λ phage DNA has identified fragment four (27 residues) as the binding peptide. Further fragmentation and fractionation of this tryptide is currently in progress to more accurately identify the nature of the binding between repressor and DNA. (Steers)

SECTION ON PROTEIN CONFORMATION

I. Evolution and Dynamics of Cytochrome c

A. Chemical and Enzymatic Synthesis

It is hypothesized that the specific cooperative interactions operative throughout the three-dimensional structure evolve in the late phase of folding to stabilize structure and that the mechanism of this global cooperative interactions, also possibly related to the function of cytochrome c, would have been conserved throughout evolution for 1.5 billion years. Thus, testing this hypothesis by synthesis of the ancestor cytochrome c would be an effective approach to the study of the relationship between the amino acid sequence (translated from the genetic information) and the structure-function of proteins. Yeast mitochondrial cytochrome c synthetase which catalyzes the covalent attachment of the heme group to apocytochrome c to form cytochrome c has now been solubilized using Triton X-100. The product formed by the action of the solubilized enzyme from hemin and apoprotein in the presence of NADPH was isolated. The isolated product exhibited Soret absorbance bands identical with those of cytochrome c, indicating the presence of two thio-ether bonds. It is also found that the enzyme recognizes the chemically synthesized fragment containing residues 1 to 25 as a substrate. Thus, in principle, the way for total synthesis of the ancestor cytochrome c is now opened. (Taniuchi, Veloso, Visco)

B. Conformational Dynamics

The motion of the three-fragment complex consisting of heme fragment (1-25)H and apofragments (28-38) and (56-104) of horse cytochrome c reported in the previous year is interpreted as indicating that the transition occurs between the different energy states of the complex without a large change in conformation by modulation of the global cooperative interactions which evolve in the late phase of folding to stabilize the structure. Furthermore, the complex does not seem to remember the pathway through which it has folded and can unfold through reversal of any folding pathways depending on the solution conditions. Replacement of evolutionarily invariant leucine 32 even with similar non-polar residues such as isoleucine, valine, and norvaline results in a drastic decrease in the binding force of the three-fragment complex. On the other hand, evolutionarily variant leucine 35 can be replaced with isoleucine without disrupting the binding force. These observations indicate that the mechanism for the global cooperative interactions is highly specific and related to the mechanism of increasing the oxidation-reduction potential to the level of cytochrome _c_. Thus, the mechanism seems to be crucial not only for folding but also for the function of cytochrome _c_ and conserved through evolution for 1.5 billion years. (Taniuchi, Poerio, Pandin)

C. Folding Mechanism

To approach to the goal of predicting the three-dimensional structure on the basis of the amino acid sequence translated from the genetic information, we investigate the global cooperative interactions which evolve only in the late phase of folding to stabilize the structure. The kinetic and thermodynamic studies of folding of the fragment complexes from horse cytochrome _c_ and the kinetic studies of folding of the fragment complexes from tuna cytochrome _c_ have indicated that 1) the native or native-like complexes can assume different energy states 2) the transition between these energy states may occur by modulation of the global cooperative interactions without a large change in conformation (no large change in the environment of non-polar groups) 3) this transition is thermodynamically characterized by a large change in entropy and enthalpy (entropy-enthalpy compensation) 4) the reduction-oxidation potential of cytochrome _c_ may also be modulated by change of the global cooperative interactions. Similar conclusions have also been reached by studies of the conformational dynamics of biologically active three-fragment complex of cytochrome _c_. (Taniuchi)

SECTION ON MACROMOLECULAR BIOLOGY

I. Normal and Abnormal Hemoglobins

A. Intracellular Polymerization of Sickle Hemoglobin

Intracellular polymerization of deoxyhemoglobin S is the basis for the pathophysiology of sickle cell anemia. We found that intracellular polymer can be detected at high oxygen saturation (greater than 90%) in SS erythrocytes as measured by double nuclear magnetic resonance and increased with decreasing oxygen saturation, in general agreement with theoretical calculations. Intracellular polymer is now being measured in SS erythrocytes separated on the basis of corpuscular hemoglobin concentration. The small difference in intracellular polymer formation between whole SS cell populations and the more uniform fractionated subpopulation at the same MCHC represents the contribution from cell heterogeneity. In our studies on the other sickle syndromes, polymer in SC erythrocytes is detected below 70% oxygen saturation, following more closely the behavior of AS than SS erythrocytes. Although hemoglobin C behaves like hemoglobin A in copolymerization with hemoglobin S, the relative clinical severity of SC disease compared with benign AS can be attributed to an increase in MCHC with a substantial increase of cells with MCHC greater than 40 g/dl. Further, our polymer calculations for the sickle syndromes correlates well with hemolytic anemia as measured by total hemoglobin. (Schechter, Noguchi)

B. Electron Microscopic Studies of Intracellular Polymerization

We are using electron microscopy to study the amount and physical properties of the polymer of deoxyhemoglobin S that forms in sickle erythrocytes upon deoxygenation. The electron microscopic studies are being correlated with results from nuclear magnetic resonance spectroscopy and other methods to examine the physical, physiological and clinical variables that affect intracellular polymerization. These studies will allow us to test various hypotheses about the pathophysiology of sickle cell disease. We are also studying diphosphoglyceric acid analogs as possible inhibitors of deoxyhemoglobin S gelation and to clarify the transport of these compounds into the erythrocyte. (Schechter, Acquaye, Noguchi)

C. Design and Synthesis of Gelation Inhibitors

Inhibition of deoxyhemoglobin S gelation by noncovalent modifiers such as phenylalanine is one strategy for potential therapeutic intervention of sickle cell anemia. Several synthetic ring substituted and other phenylalanine analogs have been tested for their inhibitory potential using equilibrium solubility and kinetic assays of sickle hemoglobin gelation. Ring substituted compounds such as o-methylphenylalanine and p-aminophenylalanine retained the inhibitory effect similar to phenylalanine. Compounds such as m-nitrophenylalanine and o-nitrophenylalanine methyl ester had no inhibitory effect of deoxyhemoglobin S gelation. The stereospecific interaction of these inhibitors with hemoglobin is being studied by X-ray diffraction/difference Fourier methods using co-crystalization of these and similar compounds with R state and T state hemoglobins in several solvent systems. (Schechter, Noguchi)

D. Phase Transitions in Sickle Hemoglobin Polymerization

We have characterized thermodynamically the polymerization and gelation of deoxygenated sickle cell hemoglobin at four temperatures and have calculated entropies and enthalpies of gelation. From measurements of chemical potentials beyond the gel phase transition over a wide concentration range we can separate for the first time the work of polymerization from that of concentrating and orienting gel fibers. Osmotic stress studies should yield not only information about the efficacy of gelation-inhibiting agents but clues as to their mechanism of action and about intermolecular interaction energies. Theories of polymerization and alignment of deoxyhemoglobin S can not only be tested by osmotic stress results, but such thermodynamic data can be used to guide theoretical development of models for the gelation process. (Schechter, Prouty)

II. Erythroid Cell Biology and Molecular Genetics

A. Regulation of Hemoglobin Synthesis

We are studying control of expression of globin genes by using as a model system, the K562 human leukemia cell line. We previously showed that these primitive cells can be reversibly induced by hemin to accumulate large amounts of embryonic and fetal hemoglobins in the absence of erythroid differentiation. Hybridization of nick-translated ^{32}P-globin cDNA probes to agarose gel blots of RNA showed increased globin mRNA levels in induced cells. Quantitation by isolation and in vitro translation of mRNA for the α, γ, ε and ζ globins expressed in these cells has shown several-fold chain specific increases after induction. This suggests that at least part of the effect of hemin is exerted at the transcriptional level. No evidence of β-globin production by K562 cells has been obtained which indicates the line may be developmentally arrested at an embryonic or fetal level. Preliminary data suggest that this lack of expression is not due to hypermethylation of β-globin DNA. (Schechter, A. Dean, Fordis, Wu)

B. Globin Gene Expression

An assay system is being developed in the K562 cell 1) to evaluate the effect of trans-acting elements on globin gene expression, 2) to permit the simultaneous analysis of transcriptional and translational controls on the differential expression of genes such as β and δ globin and 3) to investigate gene activation in what may be a "developmentally arrested" cell. Initially, we are attempting to use the K562 cell as a transient expression system. K562 cells have been transfected with PLTN3BLβ, a plasmid which contains the β gene, the two SV40 72bp repeats, and the SV 40 origin of replication. The total RNA has been isolated and is now being subjected to S_1 analysis. If β specific message is found, the production of protein will be examined as will the effect of transfected globin genes, pseudogenes, and intragenic segments on K562 globin gene expression. To determine that the β gene (which is not expressed in K562 cells) is intact, Eco RI and Hind III libraries are being constructed and the cloned β gene will be placed into a heterologous expression system to study β message. To facilitate long term studies of gene expression, a TK^- K562 subclone is being sought by subjecting K562 cells to increasing concentrations of bromodeoxyuridine. (Schechter, Fordis, A. Dean, Wu)

C. Hemoglobin Degradation

We are investigating the mechanism of hemoglobin degradation in erythroid cells. The systems under study are K562 cells, reticulocytes, and red cells infected with malarial parasites. We plan to identify degradative products as well as any critical proteinases or cofactors, and gain some understanding of the molecular mechanism of protein degradation in normal and diseased cells. Our data suggests that the acid proteolytic activity previously observed in extracts of the mouse malarial parasite P. berghei is actually of red cell origin. A parasitic activity is revealed, however, upon activation with dithiothreitol and is apparently responsible for hemozoin production. A corresponding proteinase has also been identified and partially purified from the human malarial parasite P. falciparum. In studies of the K562 human erythroleukemic cell line, we have found that uninduced and hemin-induced cells have similar levels of a cathepsin D enzyme. (Schechter, Friedman)

III. Early Mammalian Development

A. Gene Expression in the Oocyte and Embryo

We are investigating gene regulation in early mammalian development using the synthesis of the zona pellucida and proteoglycans as model systems. Using a murine follicle culture we have demonstrated the in vitro biosynthesis of the zona and calculated its turnover and rate of synthesis. The three sulfate containing glycoproteins of the zona have marked size and charge heterogeneity. Pre-incubation with tunicamycin inhibits the secretion of the zona proteins although their intracellular presence can be demonstrated by immunoprecipitation with antizona antibodies. The synthesis of zona proteins terminates with oocyte maturation prior to ovulation. Proteoglycans, however, are not produced during oogenesis and their synthesis begins only in the early embryo. Using our follicle culture we have demonstrated that although intact proteoglycans are synthesized in the surrounding granulosa cells, no proteoglycan or glycoside transferase activity is present either during oogenesis or in superovulated eggs. We have initiated studies to isolate intact proteoglycans from the zygote, the morulae and the early blastocyst stages of the mouse embryo. These macromolecules will be analyzed both as to their composition, biosynthesis and relevant biological role in pre-implantation development. (J. Dean, Tsuji)

B. Immunological Studies of the Zona Pellucida

The zona pellucida is a glycocalyx that surrounds maturing oocytes. It is actively synthesized during oogenesis until just prior to ovulation. A method has been devised for large scale isolation of the zona and subsequent purification of its three component glycoproteins. Particulate zonae were used to immunize male rats. The resulting polyclonal antibodies specifically immunoprecipitated all three of the ^{125}I-zona proteins and bound to the zona of oocytes and early embryos as demonstrated by indirect immunofluorescence labelling utilizing a fluorescein conjugated sheep anti-rat Ig antibody. Spleen cells from the same rats were fused with murine SP2/0 myeloma cells and resulting hybridomas were selected in HAT media. Several clones secreted monoclonal antibodies which are specific for the zona pellucida glycoproteins. These specific immunological probes are being used to study the supramolecular structure of the zona pellucida during development. They also will be used to immunoprecipitate _in vitro_ translation products to determine the availability of zona specific mRNA's during development. (J. Dean, East)

C. Processing of Sperm Nuclei

We are investigating the mechanisms by which the mammalian egg processes sperm chromatin prior to the expression of the male genome in early embryogenesis. During spermatogenesis the somatic histones are replaced by cysteine-rich protamines which form the nucleus into a morphologically dense structure. When isolated, these nuclei are extraordinarily resistant to chemical, physical and enzymatic attack. However, upon penetration into the egg's cytoplasm, the nuclei are rapidly processed in a series of reactions that include the reduction of disulfide bonds, the decondensation of the nuclei and the reconstitution of the DNA onto somatic histones. Using endonucleases as probes, we have demonstrated _in vitro_ that after reduction and alkylation of purified sperm nuclei, we can mimic the decondensation and reconstitution steps with a polyacidic protein. We hope to assay the oocytes for the macromolecules that mediates these reactions _in vivo_. (J. Dean)

SMITHSONIAN SCIENCE INFORMATION EXCH GE PROJECT NUMBER (Do **NOT** use this spac	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 25,000-09 LCB

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Synthesis and Characterization of Fragments of Human Leukocyte α Interferon

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M.E. Smith	Staff Fellow	LCB NIADDK
OTHERS:	M. Ohno	Visiting Scientist	LCB NIADDK
	F. Widmer	Visiting Scientist	LCB NIADDK
	N. Nelson	Chemist	LCB NIADDK

COOPERATING UNITS (if any)

Weizmann Institute of Science and The Johns Hopkins University
(Dr. C.B. Anfinsen)

LAB/BRANCH

Laboratory of Chemical Biology

SECTION

Section on Protein Chemistry

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.2	2.2	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

For elucidation of structure and function relationships in human leukocyte interferon αA and αD, a number of peptides from the C-terminal region were synthesized by conventional solution synthesis and solid phase synthesis and their biological and immunological characteristics were investigated. The peptide 121-166 was shown to contain antigenic determinants and used to produce antibodies specific for native leukocyte interferon. Analysis using smaller peptides resulted in localization of two distinct determinants in this sequence . Assays of antiviral activity and cellular membrane receptor binding are in progress.

Attempts to develop further the methodology of enzymic peptide synthesis showed that trypsin - in the presence of methylamine - catalysed formation of peptide bonds of nonspecific amino acid residues such as glycine, alanine, serine, valine and histidine. Further, we have introduced two novel α-amino protecting groups compatible with enzymic coupling in aqueous phase: the acid labile citraconyl group and the phenylacetyl group which can be cleaved enzymatically with penicillinacylase. These new techniques will eventually be applied to the synthesis of other highly pure peptides of biological interest.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 25,001-08 LCB

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

The Surface Antigens of Paramecia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. Steers	Research Biologist	LCB NIADDK
OTHERS:	A. Barnett	Guest Worker	LCB NIADDK
	C.E. Lee	Physical Science Technician	LCB NIADDK

COOPERATING UNITS (if any)

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INSTITUTE AND LOCATION

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.1	0.2

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

An array of specific serotypes have been identified within the species Paramecium tetraurelia on the basis of specific responses to diverse immune sera. This response is the result of a surface localized antigen (i-antigen) which covers the entire cell except for a region along the oral suture (midline). Numerous studies have been carried out on the surface i-antigens, but these studies have been restricted in every case to those of a single species - P. tetraurelia. This project involves the identification, purification and characterization of the surface antigens from another species, P. multimicronucleatum.

This project is inactive at the present time.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 25,002-09 LCB

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

The Hemoglobins of Paramecia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. Steers, Jr.	Research Biologist	LCB NIADDK
OTHERS:	A. Barnett	Guest Worker	LCB NIADDK
	C.E. Lee	Physical Science Technician	LCB NIADDK

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Chemical Biology

SECTION

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INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

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0	0	0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is inactive.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 25,003-12 LCB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Semisynthesis of Proteins

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. Kanmera	Visiting Fellow	LCB NIADDK
OTHERS:	I.M. Chaiken	Research Chemist	LCB NIADDK

COOPERATING UNITS (if any)
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Laboratory of Chemoprevention, NCI
Instituto di Chimica Organica, Universita di Padova

LAB/BRANCH
Laboratory of Chemical Biology

SECTION
Section on Protein Chemistry

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Md. 20205

TOTAL MANYEARS: 1.3	PROFESSIONAL: 1.3	OTHER: 0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is part of the continuing studies, using protein semisynthesis to understand the relationship between primary structure of proteins and the higher order properties of folding, interactions, and ultimate biological functions. Sequence modeling studies of ribonuclease have been undertaken, which involve the design and the chemical semisynthesis of model proteins with specific chemical and conformational properties directed artificially. The studies of semisynthetic model proteins have provided the insight into the mode of non-covalent interaction between RNase-(1-20) and RNase-(21-124).

The studies of neurophysins and their precursors is also progressing through their semisynthesis in order to investigate hormone-protein interaction, folding of proteins, and biosynthetic pathway of hormones.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 25,004-09 LCB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Peptide-Protein Interaction: Studies by Quantitative Affinity Chromatography

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	David M. Abercrombie	Chemist	LCB NIADDK
OTHERS:	Irwin M. Chaiken	Research Chemist	LCB NIADDK
	Daniele Dalzoppo	Guest Worker	LCB NIADDK

COOPERATING UNITS (if any)

Istituto di Chimica Organica, Universita di Padova

LAB/BRANCH
Laboratory of Chemical Biology

SECTION
Section on Protein Chemistry

INSTITUTE AND LOCATION
NIADDK, Bethesda, Maryland 20205

TOTAL MANYEARS: 1.1	PROFESSIONAL: 1.1	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is part of a continuing program to develop the use of quantitative affinity chromatography for the characterization of macromolecular interactions. As part of the current studies, adsorbents containing immobilized neuropeptide hormones and their analogues are being used to elucidate the binding interactions and ligand-induced self-association of bovine neurophysins (NP's). The quantitative chromatographic behavior observed with native NPII, and NPII, mononitrated at Tyr 49 to eliminate monomer bivalency, were compared. The chromatographic approach has been used in project 25,006 to study the dependence of hormone-mediated NP self-association on the intactness of the hormone binding surface. This method also is being used to study the nature of interactions of active site peptide ligands with polypeptide fragment complexes of the thermostable protease, thermolysin.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 25,006-09 LCB

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Chemical Studies of Neurophysin-Neurohypophyseal Hormone Complexes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	David M. Abercrombie	Chemist	LCB NIADDK
OTHERS:	Irwin M. Chaiken	Research Chemist	LCB NIADDK
	Reginald P. Sequeira	Visiting Fellow	LCB NIADDK

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Chemical Biology

SECTION

Section on Protein Chemistry

INSTITUTE AND LOCATION

NIADDK, Bethesda, Maryland 20205

TOTAL MANYEARS: 0.7	PROFESSIONAL: 0.7	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We have been investigating the structural requirements in neurophysins (NP's) for hormone binding, self-association, and antibody recognition. The principal approach during this period has been to determine the alteration in these properties in NP's that have been derivatized. Bovine NP derivatized by trypsin digestion and photo-affinity labelling of the hormone binding site were analyzed by affinity chromatography and radioimmunoassay. These data were correlated with radioimmunoassay data for disulfide-broken NP's and native NP's with different sequences. Overall, the results showed that NP hormone binding and self-association are critically dependent upon a structurally intact protein molecule. In contrast, antibody recognition is lost only in NP derivatives in which disulfide bonds are broken.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 25,007-09 LCB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Biosynthetic Origin of Neurophysin-Neuropeptide Hormone Complexes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Reginald P. Sequeira	Visiting Fellow	LCB NIADDK
OTHERS:	Irwin M. Chaiken	Research Chemist	LCB NIADDK

COOPERATING UNITS (if any)
Adi F. Gazdar, Navy Medical Oncology, NCI

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Laboratory of Chemical Biology

SECTION
Section on Protein Chemistry

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Md. 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The project is a part of a general program investigating biochemical aspects of biosynthesis of neurophysins and neurohypophysial hormones, with a current emphasis on ectopic hormone-protein synthesis by tumors. The human tumor cell lines of small cell carcinoma of lung (SCCL) is a good model to study the relationship between tumor biology and ectopic peptide/protein biosynthesis. An attempt has been made to grow SCCL in vitro and screen them for the presence of neurohypophysial peptide hormones and neurophysins. Special attention will be focussed to elucidate the biochemical and immunological nature of vasopressin-neurophysin precursor(s). The understanding of ectopic hormone and protein biosynthesis by tumors may shed light on the nature of cellular differentiation and the process of malignant changes. Such a study also raises the possiblity of presymptomatic detection of small cell carcinoma of lung and the precise assessment of therapy.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 25,008-19 LCB

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Biological and Chemical Synthesis of Cytochrome c

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Hiroshi Taniuchi	Chief, Section on Protein Conformation	LCB NIADDK
OTHERS:	Dulce Veloso	Visiting Fellow	LCB NIADDK
	Carlo Visco	Visiting Fellow	LCB NIADDK

COOPERATING UNITS (if any)

University of Padova, Padova, Italy

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Laboratory of Chemical Biology

SECTION

Protein Conformation Section

INSTITUTE AND LOCATION

NIADDK, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.7	2.3	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

It is hypothesized that the specific cooperative interactions operative throughout the three-dimensional structure evolve in the late phase of folding to stabilize structure and that the mechanism of this global cooperative interactions, also possibly related to the function of cytochrome c, would have been conserved throughout evolution for 1.5 billion years. Thus, testing this hypothesis by synthesis of the ancestor cytochrome c would be an effective approach to the study of the relationship between the amino acid sequence (translated from the genetic information) and the structure-function of proteins. Yeast mitochondrial cytochrome c synthetase which catalyzes the covalent attachment of the heme group to apocytochrome c to form cytochrome c (see the previous report) has now been solubilized using Triton X-100. The product formed by the action of the solubilized enzyme from hemin and apoprotein in the presence of NADPH was isolated. The isolated product exhibited Soret absorbance bands identical with those of cytochrome c, indicating the presence of two thio ether bonds. It is also found that the enzyme recognizes the chemically synthesized fragment containing residues 1 to 25 as a substrate. Thus, in principle, the way for total synthesis of the ancestor cytochrome c is now opened.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 25,011-08 LCB

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

The Mechanism of Protein Folding: Conformational Dynamics

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Hiroshi Taniuchi	Chief, Section on Protein Conformation	LCB NIADDK
OTHERS:	Elia Poerio	Visiting Associate	LCB NIADDK
	Mario Pandin	Visiting Associate	LCB NIADDK

COOPERATING UNITS (if any)

Department of Biochemistry, University of Geneva, Geneva, Switzerland

LAB/BRANCH
Laboratory of Chemical Biology

SECTION
Section on Protein Conformation

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	1.1	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The motion of the three-fragment complex consisting of heme fragment (1-25)H and apofragments (28-38) and (56-104) of horse cytochrome *c* reported in the previous year is interpreted as indicating that the transition occurs between the different energy states of the complex without a large change in conformation by modulation of the global cooperative interactions which evolve in the late phase of folding to stabilize the structure. Furthermore, the complex does not seem to remember the pathway through which it has folded and can unfold through reversal of any folding pathways depending on the solution conditions. Replacement of evolutionarily invariant leucine 32 even with similar non-polar residues such as isoleucine, valine, and norvaline results in a drastic decrease in the binding force of the three-fragment complex. On the other hand, evolutionarily variant leucine 35 can be replaced with isoleucine without disrupting the binding force. These observations indicate that the mechanism for the global cooperative interactions is highly specific and related to the mechanism of increasing the oxidation-reduction potential to the level of cytochrome *c*. Thus, the mechanism seems to be crucial not only for folding but also for the function of cytochrome *c* and conserved through evolution for 1.5 billion years.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 25,016-09 LCB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Regulation of Hemoglobin Synthesis in Cultured Erythroid Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Ann Dean	Research Chemist	LCB NIADDK
OTHERS:	Michael Fordis	Medical Staff Fellow	LCB NIADDK
	Yongji Wu	Guest Worker	LCB NIADDK
	Alan N. Schechter	Chief, Laboratory of Chemical Biology	LCB NIADDK

COOPERATING UNITS (if any)
Drs. Richard Klausner, Kenneth Bridges, LBM, NIADDK.
Dr. Mark Smulson, Department of Biochemistry, Georgetown University.

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SECTION
Section on Macromolecular Biology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We are studying control of expression of globin genes by using as a model system, the K562 human leukemia cell line. We previously showed that these primitive cells can be reversibly induced by hemin to accumulate large amounts of embryonic and fetal hemoglobins in the absence of erythroid differentiation. Hybridization of nick-translated 32P-globin cDNA probes to agarose gel blots of RNA showed increased globin mRNA levels in induced cells. Quantitation by isolation and in vitro translation of mRNA for the α, γ, ε and ζ globins expressed in these cells has shown several-fold chain specific increases after induction. This suggests that at least part of the effect of hemin is exerted at the transcriptional level. No evidence of β-globin production by K562 cells has been obtained which indicates the line may be developmentally arrested at an embryonic or fetal level. Preliminary data suggest that this lack of expression is not due to hypermethylation of β-globin DNA.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 25,021-07 LCB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Sickle Cell Anemia: The Intracellular Polymerization of Hemoglobin S

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C.T. Noguchi	Staff Fellow	LCB NIADDK
OTHERS:	A.N. Schechter	Chief, Laboratory of Chemical Biology	LCB NIADDK

COOPERATING UNITS (if any)
NIDR - Dr. Dennis Torchia; NIADDK - Dr. William Eaton; NHLBI - Dr. Arthur Nienhuis; BEIB - Dr. David Markle; Harvard Medical School, Boston, MA - Dr. H. Frank Bunn; Case-Western Reserve Medical School, Cleveland, OH - Dr. Gary Brittenham

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SECTION
Section on Macromolecular Biology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.9	0.9	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Intracellular polymerization of deoxyhemoglobin S is the basis for the pathophysiology of sickle cell anemia. We found that intracellular polymer can be detected at high oxygen saturation (greater than 90%) in SS erythrocytes as measured by double nuclear magnetic resonance and increased with decreasing oxygen saturation, in general agreement with theoretical calculations. Intracellular polymer is now being measured in SS erythrocytes separated on the basis of corpuscular hemoglobin concentration. The small difference in intracellular polymer formation between whole SS cell populations and the more uniform fractionated subpopulation at the same MCHC represents the contribution from cell heterogeneity. In our studies on the other sickle syndromes, polymer in SC erythrocytes is detected below 70% oxygen saturation, following more closely the behavior of AS than SS erythrocytes. Although hemoglobin C behaves like hemoglobin A in copolymerization with hemoglobin S, the relative clinical severity of SC disease compared with benign AS can be attributed to an increase in MCHC with a substantial increase of cells with MCHC greater than 40 g/dl. Further, our polymer calculations for the sickle syndromes correlates well with hemolytic anemia as measured by total hemoglobin.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 25,023-06 LCB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Role of Surface Molecules in Cell Recognition and Communication.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. Steers, Jr.	Research Biologist	LCB NIADDK
OTHERS:	A. Kitamura	Guest Worker	LCB NIADDK
	C.E. Lee	Physical Science Tech.	LCB NIADDK

COOPERATING UNITS (if any)

Dr. A.J. Barnett, Department of Biology, University of Maryland, College Park.

LAB/BRANCH
Laboratory of Chemical Biology

SECTION
Protein Chemistry Section

INSTITUTE AND LOCATION
NIADDK, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.2	0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

When paramecia are placed in homologous immune sera they become immobilized and eventually die. This specific reaction has been shown to be due to a series of protein antigens associated with the cell surface. Fluorescence studies reveal a pattern of Ag-Ab redistribution over the cell surface analogous to the capping phenomenon reported in other cell types. The process of redistribution occurs at the tips of the cilia and frequently results in the agglutination of several cilia into groups. TEM studies on sections through the ciliary bundles of immobilized cells show the axonemes of these agglutinated cilia contained within a single unit membrane. This fusion of ciliary membranes is readily induced by intact immune IgG, but is not induced by Fab fragments prepared from the same IgG. Cross-linking of the Fab fragments with goat anti-rabbit IgG results in immobilization and membrane fusion identical to that seen with intact immune IgG.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 25,025-06 LCB

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

A Study of the Folding Mechanism of Cytochrome c Using Complementing Fragments

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Hiroshi Taniuchi	Chief, Section on Protein Conformation	LCB NIADDK
OTHERS:	Gary R. Parr	Senior Staff Fellow	LCB NIADDK

COOPERATING UNITS (if any)

Department of Biochemistry, Clemson University, South Carolina

LAB/BRANCH
Laboratory of Chemical Biology

SECTION
Section on Protein Conformation

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0.4	PROFESSIONAL: 0.4	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

To approach to the goal of predicting the three-dimensional structure on the basis of the amino acid sequence translated from the genetic information, we investigate the global cooperative interactions which evolve only in the late phase of folding to stabilize the structure. The kinetic and thermodynamic studies of folding of the fragment complexes from horse cytochrome c and the kinetic studies of folding of the fragment complexes from tuna cytochrome c have indicated that 1) the native or native-like complexes can assume different energy states 2) the transition between these energy states may occur by modulation of the global cooperative interactions without a large change in conformation (no large change in the environment of non-polar groups) 3) this transition is thermodynamically characterized by a large change in entropy and enthalpy (entropy-enthalpy compensation) 4) the reduction-oxidation potential of cytochrome c may also be modulated by change of the global cooperative interactions. Similar conclusions have also been reached by studies of the conformational dynamics of biologically active three-fragment complex of cytochrome c (see another report from this section).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 25,026-05 LCB

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

The Solubility of Reconstituted Hemoglobins A and S in High Phosphate

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	K. Alston	Staff Fellow	LCB NIADDK
	F.K. Friedman	Staff Fellow	LCB NIADDK
	A.N. Schechter	Chief, Laboratory of Chemical Biology	LCB NIADDK

COOPERATING UNITS (if any)

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Laboratory of Chemical Biology

SECTION

Section on Macromolecular Biology

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0	PROFESSIONAL: 0	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Terminated.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 25,028-04 LCB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Sickle Cell Anemia: Design and Synthesis of Gelation Inhibitors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C.T. Noguchi	Senior Staff Fellow	LCB NIADDK
OTHERS:	A.N. Schechter	Chief, Laboratory of Chemical Biology	LCB NIADDK

COOPERATING UNITS (if any) Division of Computer Research and Technology, NIH (Dr. R.J. Feldmann); Chemical Research Institute, Montreal, Canada (Dr. P. Schiller); Medical Research Council Laboratory of Molecular Biology, Cambridge, England (Drs. S.E.V. Phillips and M.F. Perutz)

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Laboratory of Chemical Biology

SECTION
Section on Macromolecular Biology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Inhibition of deoxyhemoglobin S gelation by noncovalent modifiers such as phenylalanine is one strategy for potential therapeutic intervention in sickle cell anemia. Several synthetic ring substituted and other phenylalanine analogs have been tested for their inhibitory potential using equilibrium solubility and kinetic assays of sickle hemoglobin gelation. Ring substituted compounds such as o-methylphenylalanine and p-aminophenylalanine retained the inhibitory effect similar to phenylalanine. Compounds such as m-nitrophenylalanine and o-nitrophenylalanine methyl ester had no inhibitory effect on deoxyhemoglobin S gelation. The stereospecific interaction of these inhibitors with hemoglobin is being studied by X-ray diffraction/difference Fourier methods using co-crystalization of these and similar compounds with R state and T state hemoglobins in several solvent systems.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 25,029-04 LCB

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Early Mammalian Development: Gene Expression in the Maturing Oocyte and Embryo

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. Tsuji	Visiting Fellow	LCB NIADDK
OTHERS:	J. Dean	Senior Investigator	LCB NIADDK

COOPERATING UNITS (if any)

W. Lennarz, D. Carson, Dept. of Physiological Chemistry, The Johns Hopkins University, Baltimore, Maryland

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Laboratory of Chemical Biology

SECTION

Section on Macromolecular Biology

INSTITUTE AND LOCATION

NIADDK, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We are investigating gene regulation in early mammalian development using the synthesis of the zona pellucida and proteoglycans as model systems. Using a murine follicle culture we have demonstrated the in vitro biosynthesis of the zona and calculated its turnover and rate of synthesis. The three sulfate containing glycoproteins of the zona have marked size and charge heterogeneity. Pre-incubation with tunicamycin inhibits the secretion of the zona proteins although their intracellular presence can be demonstrated by immunoprecipitation with antizona antibodies. The synthesis of zona proteins terminates with oocyte maturation prior to ovulation. Proteoglycans, however, are not produced during oogenesis and their synthesis begins only in the early embryo. Using our follicle culture we have demonstrated that although intact proteoglycans are synthesized in the surrounding granulosa cells, no proteoglycan or glycoside transferase activity is present either during oogenesis or in superovulated eggs. We have initiated studies to isolate intact proteoglycans from the zygote, the morulae and the early blastocyst stages of the mouse embryo. These macromolecules will be analyzed both as to their composition, biosynthesis and revelant biological role in pre-implantation development.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 25,031-04 LCB

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Preparation of High Specificity Antisera to Human Interferon and Synthetic Interferon Fragments.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. Smith	Staff Fellow	LCB NIADDK
OTHERS:	N. Nelson	Chemist	LCB NIADDK

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Chemical Biology

SECTION

Section on Protein Chemistry

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0	PROFESSIONAL: 0	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Terminated.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 25,034-03 LCB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Early Mammalian Development: Processing of Sperm Nuclei by Mouse Oocytes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Jurrien Dean	Senior Investigator	LCB NIADDK

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Chemical Biology

SECTION
Section on Macromolecular Biology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.6	.6	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We are investigating the mechanisms by which the mammalian egg processes sperm chromatin prior to the expression of the male genome in early embryogenesis. During spermatogenesis the somatic histones are replaced by cysteine-rich protamines which form the nucleus into a morphologically dense structure. When isolated, these nuclei are extraordinarily resistant to chemical, physical and enzymatic attack. However, upon penetration into the egg's cytoplasm, the nuclei are rapidly processed in a series of reactions that include the reduction of disulfide bonds, the decondensation of the nuclei and the reconstitution of the DNA onto somatic histones. Using endonucleases as probes, we have demonstrated in vitro that after reduction and alkylation of purified sperm nuclei, we can mimic the decondensation and reconstitution steps with a polyacidic protein. We hope to assay the oocytes for the macromolecules that mediates these reactions in vivo.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 25,035-03 LCB

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Nuclear Magnetic Resonance Spectroscopy of Human Erythrocytes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S.K. Ackerman	Guest Worker	LCB NIADDK
OTHERS:	C.T. Noguchi	Senior Staff Fellow	LCB NIADDK
	A.N. Schechter	Chief, Laboratory of Chemical Biology	LCB NIADDK

COOPERATING UNITS (if any)

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Laboratory of Chemical Biology

SECTION

Section on Macromolecular Biology

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0	PROFESSIONAL: 0	OTHER: 0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is inactive at this time.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 25,036-03 LCB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Osmotic Stress Response of Deoxygenated Sickle Hemoglobin in Solutions and Gels

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Muriel Prouty	Guest Worker	LCB NIADDK
OTHERS:	Alan N. Schechter	Chief, Laboratory of Chemical Biology	LCB NIADDK

COOPERATING UNITS (if any)
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LAB/BRANCH
Laboratory of Chemical Biology

SECTION
Section on Macromolecular Biology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	1.1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We have characterized thermodynamically the polymerization and gelation of deoxygenated sickle cell hemoglobin at four temperatures and have calculated entropies and enthalpies of gelation. From measurements of chemical potentials beyond the gel phase transition over a wide concentration range we can separate for the first time the work of polymerization from that of concentrating and orienting gel fibers. Osmotic stress studies should yield not only information about the efficacy of gelation-inhibiting agents but clues as to their mechanism of action and about intermolecular interaction energies. Theories of polymerization and alignment of deoxyhemoglobin S can not only be tested by osmotic stress results, but such thermodynamic data can be used to guide theoretical development of models for the gelation process.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 25,037-03 LCB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Mechanism of Hemoglobin Degradation in Erythroid Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	F.K. Friedman	Staff Fellow	LCB NIADDK
OTHERS:	A.N. Schechter	Chief, Laboratory of Chemical Biology	LCB NIADDK

COOPERATING UNITS (if any)
Laboratory of Parasitic Diseases, NIAID (Drs. T. Hadley and L. Miller)

LAB/BRANCH
Laboratory of Chemical Biology

SECTION
Section on Macromolecular Biology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Md. 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	1.1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We are investigating the mechanism of hemoglobin degradation in erythroid cells. The systems under study are K562 cells, reticulocytes, and red cells infected with malarial parasites. We plan to identify degradative products as well as any critical proteinases or cofactors, and gain some understanding of the molecular mechanism of protein degradation in normal and diseased cells. Our data suggests that the acid proteolytic activity previously observed in extracts of the mouse malarial parasite *P. berghei* is actually of red cell origin. A parasitic activity is revealed, however, upon activation with dithiothreitol and is apparently responsible for hemozoin production. A corresponding proteinase has also been identified and partially purified from the human malarial parasite *P. falciparum*. In studies of the K562 human erythroleukemic cell line, we have found that uninduced and hemin-induced cells have similar levels of a cathepsin D enzyme.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 25,038-02 LCB

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

The K562 Cell Line: An Assay System for Globin Gene Expression

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. Michael Fordis	Medical Staff Fellow	LCB NIADDK
OTHERS:	Ann Dean	Research Chemist	LCB NIADDK
	Yonji Wu	Guest Worker	LCB NIADDK
	Alan N. Schechter	Chief, Laboratory of Chemical Biology	LCB NIADDK

COOPERATING UNITS (if any)

Clinical Hematology Branch, NHLBI: Dr. Arthur W. Nienhuis, Dr. Keith Humphries, Dr. Rick Anagnou, Dr. Tim Ley

LAB/BRANCH

Laboratory of Chemical Biology

SECTION

Section on Macromolecular Biology

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Md 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.4	1.4	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

An assay system is being developed in the K562 cell 1) to evaluate the effect of trans-acting elements on globin gene expression, 2) to permit the simultaneous analysis of transcriptional and translational controls on the differential expression of genes such as β and δ globin and 3) to investigate gene activation in what may be a "developmentally arrested" cell. Initially, we are attempting to use the K562 cell as a transient expression system. K562 cells have been transfected with PLTN3BLβ, a plasmid which contains the β gene, the two SV40 72bp repeats, and the SV 40 origin of replication. The total RNA has been isolated and is now being subjected to S_1 analysis. If β specific message is found, the production of protein will be examined as will the effect of transfected globin genes, pseudogenes, and intragenic segments on K562 globin gene expression. To determine that the β gene (which is not expressed in K562 cells) is intact, Eco RI and Hind III libraries are being constructed and the cloned β gene will be placed into a heterologous expression system to study β message. To facilitate long term studies of gene expression, a TK^- K562 subclone is being sought by subjecting K562 cells to increasing concentrations of bromodeoxyuridine.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 25,039-01 LCB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Early Mammalian Development: Immunological Studies of the Zona Pellucida

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	I. East	Visiting Fellow	LCB NIADDK
OTHERS:	J. Dean	Senior Investigator	LCB NIADDK

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Chemical Biology

SECTION
Section on Macromolecular Biology

INSTITUTE AND LOCATION
NIADDK, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The zona pellucida is a glycocalyx that surrounds maturing oocytes. It is actively synthesized during oogenesis until just prior to ovulation. A method has been devised for large scale isolation of the zona and subsequent purification of its three component glycoproteins. Particulate zonae were used to immunize male rats. The resulting polyclonal antibodies specifically immunoprecipitated all three of the 125I-zona proteins and bound to the zona of oocytes and early embryos as demonstrated by indirect immunofluorescence labelling utilizing a fluorescein conjugated sheep anti-rat Ig antibody. Spleen cells from the same rats were fused with murine SP2/0 myeloma cells and resulting hybridomas were selected in HAT media. Several clones secreted monoclonal antibodies which are specific for the zona pellucida glycoproteins. These specific immunological probes are being used to study the supramolecular structure of the zona pellucida during development. They also will be used to immunoprecipitate in vitro translation products to determine the availability of zona specific mRNA's during development.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 25,040-01 LCB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Cell-cell Interactions: Attachment of Paramecium to Polystyrene.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. Steers, Jr.	Research Biologist	LCB NIADDK
OTHERS:	A. Kitamura	Guest Worker	LCB NIADDK
	C.E. Lee	Physical Science Tech.	LCB NIADDK

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Chemical Biology

SECTION
Protein Chemistry Section

INSTITUTE AND LOCATION
NIADDK, Bethesda, Maryland 20205

TOTAL MANYEARS: 1.0	PROFESSIONAL: 0.8	OTHER: 0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Mating reactive cells of Paramecium caudatum attach to the polystyrene surface of certain Petri dishes. This attachment occurs at the tips of ventrally located cilia (mating reacting cells) and appears to involve a hydrophobic interaction. Although the attachment is correlated with mating reactivity, the attachment sites are distinct from the mating reactive sites. Attachment of cells to polystyrene also triggers the early migration of the gametic nucleus which precedes meiosis during the normal conjugation process. Certain aromatic compounds have been shown to greatly enhance the attachment of cells without affecting their mating reactivity.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 25,041-01 LCB

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Protein-DNA Interaction: Structure of the λ CRO Binding Site

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: E. Steers, Jr. Research Biologist LCB NIADDK

COOPERATING UNITS (if any)

Dr. Yoshinori Takeda, Chemistry Department, University of Baltimore, Catonsville

LAB/BRANCH

Laboratory of Chemical Biology

SECTION

Protein Chemistry Section

INSTITUTE AND LOCATION

NIADDK, Bethesda, Maryland 20205

TOTAL MANYEARS: 0.3	PROFESSIONAL: 0.3	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The λ CRO repressor protein has been modified by reductive methylation blocking the lysine residues to tryptic hydrolysis. Tryptic hydrolysis (restricted to arginyl residues) has resulted in four tryptides which are readily separated by HPLC. Double labelling experiments in which the methylation is carried out with either 3H or 14C formaldehyde in the presence and absence of λ phage DNA has identified fragment four (27 residues) as the binding peptide. Further fragmentation and fractionation of this tryptide is currently in progress to more accurately identify the nature of the binding between repressor and DNA.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 25,042-01 LCB

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Sickle Cell Anemia: Intracellular Polymerization of Hemoglobin S and Inhibitors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Clemenceau Acquaye	Visiting Fellow	LCB NIADDK
OTHERS:	Constance T. Noguchi	Senior Staff Fellow	LCB NIADDK
	Alan N. Schechter	Chief, Laboratory of Chemical Biology	LCB NIADDK

COOPERATING UNITS (if any)

Molecular Hematology Branch, NHLBI (Dr. Arthur W. Nienhuis and Ms. Maria Harrison)
Department of Medicine, Case-Western Reserve, Cleveland, OH (Dr. John Harris)

LAB/BRANCH

Laboratory of Chemical Biology

SECTION

Section on Macromolecular Biology

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Md. 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We are using electron microscopy to study the amount and physical properties of the polymer of deoxyhemoglobin S that forms in sickle erythrocytes upon deoxygenation. The electron microscopic studies are being correlated with results from nuclear magnetic resonance spectroscopy and other methods to examine the physical, physiological and clinical variables that affect intracellular polymerization. These studies will allow us to test various hypotheses about the pathophysiology of sickle cell disease.

We are also studying diphosphoglyceric acid analogs as possible inhibitors of deoxyhemoglobin S gelation and to clarify the transport of these compounds into the erythrocyte.

ANNUAL REPORT OF THE
LABORATORY OF PHYSICAL BIOLOGY

Muscle Contraction

X-ray diffraction techniques. Methods were developed for obtaining diffraction data from very small muscle preparations, to minimize the diffusion problems that are inherent in fiber studies, and from larger preparations in a very intense X-ray beam, to characterize the structure of short lived states. A new type of solid-state X-ray detector, made of a two dimensional array of charge-coupled devices (CCD) was obtained under contract and tested. The fabrication of this detector was the first step in the development of a large area array capable of recording the entire two dimensional X-ray diffraction pattern of muscle cells and other biological specimens with high time resolution and wide dynamic range. (Yu, Podolsky)

X-ray diffraction from relaxed muscle fibers. Equatorial X-ray diffraction from a single rabbit psoas muscle fiber (~ 100 μm in diameter) was obtained for the first time by using an X-ray camera with a fine focus ~ 50 μm in width. Ionic strength was varied between 170 mM and 19 mM at 4°C, while the fiber was kept relaxed. The intensity ratio I_{11}/I_{10} appeared to increase as a nonlinear function of decreasing ionic strength. The corresponding filament lattice spacing d_{10} decreased from 450 to 380Å. The increase in intensity ratio I_{11}/I_{10} with a decrease in ionic strength indicates a shift of mass from the thick filament region to the thin filament region. This suggests that an increasing number of crossbridges becomes attached to the thin filament at low ionic strengths, which is in close agreement with stiffness measurements described in the following section. (Brenner, Yu)

Stiffness of relaxed muscle. According to the classic steric blocking model for the regulation of muscle activity, muscle is kept relaxed in the absence of Ca^{2+} by troponin-tropomyosin blocking the attachment of myosin cross-bridges to actin. However, *in vitro*, at low ionic strength, 5°C, the binding of myosin subfragment-1 to actin-troponin-tropomyosin is not inhibited in the absence of Ca^{2+}, even though removal of Ca^{2+} inhibits the actomyosin ATPase rate. Measuring the stiffness of a relaxed rabbit psoas fiber under identical conditions (+ATP, $-Ca^{2+}$), as a function of the overlap between the actin and myosin filaments, revealed that significant numbers of crossbridges are attached to actin in the relaxed muscle at low ionic strength. This shows that a muscle can be kept relaxed by blocking of a kinetic step subsequent to attachment without blocking of the attachment step as the steric blocking model requires. It was also found that the resting stiffness decreases dramatically as the ionic strength is increased toward physiological values. In accordance with the X-ray findings, this suggests that fewer cross-bridges may be attached to actin in the resting muscle at physiological ionic strength than at low ionic strength. (Schoenberg, Brenner)

X-ray diffraction of activated muscle using synchrotron radiation. The equatorial X-ray diffraction patterns from skeletal muscle depend on the physiological state of the muscle. Following the transition from rest to full activation, the two prominent reflections [1,0] and [1,1] change in a reciprocal way. Based on these two reflections, Haselgrove and Huxley suggested that there is mass transfer from the thick filament region to the thin filament region. To improve the spatial resolution, we studied the higher orders beyond [1,1] of relaxed and fully activated frog sartorius muscle, using the intense X-ray source at Stanford Synchrotron Radiation Laboratory. The diffraction patterns were analyzed by a non-linear curve fitting procedure. Based on this analysis, the intensity of [2,0] does not change, or probably increases slightly (~ 20%) from rest to full activity, while [2,1] and [3,0] appear to decrease between 20 to 60%.

Fourier synthesis was carried out based on the mean values of the intensities. For five orders there are 32 possible phase combinations. For both the resting and active states, only two phase combinations appear to satisfy currently known results about filament masses and filament symmetry. For the resting state, the density maps indicate the presence of considerable mass (presumably myosin S1) in the region between thick and thin filaments. Upon activation, mass redistribution involves only the S1 surrounding the thick filament shaft; the shaft itself remains unchanged. This shift of mass, regardless of whether there is phase change or not upon activation, involves a significant azimuthal component. (Yu, Steven)

Properties of the rigor state. The simplest state of the actomyosin system is that in which ATP is absent. In this "rigor" condition, all the myosin heads form cross-bridges with the actin-containing filament. The force developed by a fiber in this state depends on the strain imposed upon it as well or the composition of the bathing solution. X-ray diffraction and light microscopy of the fiber structure over a wide range of ionic strength, pH, and osmotic pressures show that at low ionic strength and high pH a large fraction of the force arises from radial distortion of the normally cylindrical shape of the sarcomere. Thus part of the rigor force depends on electrostatic factors that operate at the filament rather than the cross-bridge level. This phenomenon is a potential source of information about the charge distribution along the myofilaments and the mechanical properties of both the crossbridges and the myofilaments. (Arata, Podolsky)

In vertebrate muscle at physiological sarcomere lengths (2.3 μ) the two major filaments (the actin-containing and myosin-containing filaments) always form a double hexagonal lattice. However, at long sarcomere length (3.2 μ) the equatorial X-ray reflections in the rigor state show spacings characteristic of a square rather than a hexagonal lattice. This change in myofilament lattice configuration suggests that the sarcomere may contain minor filaments in addition to the actin and myosin filaments, and that when strained these minor filaments realign the major myofilaments. (Arata, Quandt)

Cell Membranes

Permeability of lecithin surface bilayers. The structure of lecithin bilayers that form spontaneously at the air-water surface has been examined by water permeability measurements; these have been achieved by studying the rate of evaporation of water in the presence of the bilayer. At the temperature of formation of dimyristoyl lecithin surface bilayers (28.9°C) the rate of evaporation of water was decreased by approximately 50% from that of a lipid-free water surface. Raising or lowering the temprature as little as 0.1°C increased the rate of water evaporation to the value for the lipid-free system. Thus it is established that surface bilayers form in a very narrow temperature interval (0.1°C) and are a significant diffusion barrier to water. This phenomenon may have special significance with respect to the influence of temperature on membrane permeability, especially in the case of depolarizing potentials in excitable tissues. (Gershfeld)

Relation of plasma disaturated lecithin titer and coronary artery disease risk factors. A program has been developed for collecting, storing and analyzing fresh blood plasma samples from a large population. Analysis of the relation between plasma disaturated lecithin titer and the major risk factors of coronary artery disease has been initiated. (Gershfeld)

Red Cell Membrane Structure

Enzymatic hydrolysis of phospholipids located at the inner surface of the membrane bilayer had earlier been found to increase the rate of dissociation of proteins from the cytoskeletal network lining this surface. This increase has now been shown to be a result of the presence in the membrane of products of the hydrolysis, the lysophospholipids. The effects of a number of other substances on cytoskeletal protein dissociation rates were examined. Most compounds, including local anesthetics, were without effect, while only two, phosphorylcholine and 2,3 diphosphoglycerate (DPG) increased the rate. The first of these is structurally similar to the polar groups of the lysophospholipids. Further work is necessary to establish whether DPG, which is normally present in the erythrocyte, affects the binding of cytoskeletal proteins under physiological conditions. (Gottlieb)

Macromolecular structures determined by radiation inactivation of biological function

Molecular weight determinations by radiation inactivation have been performed for a variety of biological systems. All of these are complex components of membranes or organelles which cannot be easily extracted or purified by conventional methods. For example, the functional unit detected by radiation inactivation in the enzyme lipoprotein lipase from rat heart was the same for both tissues, and was not affected by starvation or feeding of the rats. The target size of 125,000 daltons is double the monomer polypeptide size known for most tissues, suggesting a dimer requirement for enzymatic activity. Sterol-related enzymes that were studied include HMG CoA reductase, enzymes controlling triglyceride synthesis, and cholesterol acyltransferase (ACAT). The first two show simple radiation inactivation, leading to target molecular weights of 250,000 and 80,000 respectively. Data from ACAT is more complex, suggesting several different-sized functional units. When this enzyme is activated by the detergent Brij, the radiation curves become simple, with only one (smaller) target size.

Several radiation studies of ligand receptors on cell membranes are in progress. The benzodiazepine receptor from rat tissues have been examined. The brain receptor shows a target size of 57,000, in excellent agreement with the monomer size seen on SDS PAGE. The functional unit is not altered in the presence of GABA. The similar receptor isolated from the kidney was shown to be a different structure, with a target size of 34,000 daltons. The beta adrenergic receptor from frog erythrocytes has been purified and shown to be composed of monomer chains of 50,000 daltons. The radiation target size was in complete agreement with this value, measured both *in situ* in the membrane and in the purified preparations.

Target analysis is also being applied to transport phenomena. The sodium-dependent glucose transporter in kidney brush border membranes was measured by phlorizin binding. A target size of 110,000 was determined, which is larger than indicated by most reports of extracted polypeptide chains believed to be associated with this transporter. It is suggested that two polymer chains are required for phlorizin binding. A different transporter, the lactose carrier from *E. coli* appears to be a monomer, since the target size and SDS PAGE both yield a value of 45,000 daltons.

Energy transfer in multi-subunit enzymes is being examined in invertase and in glutamate dehydrogenase. The latter enzyme requires a hexameric structure for activity. However, fluoresence (due to FITC covalently-bound to glutamate dehydrogenase) is lost with a target size corresponding to one monomeric unit. These data indicate that energy transfer is restricted to single chains, but the destruction on one chain can lead to a loss of enzyme activity by the entire ensemble (in this case, the hexamer) either by dissociation or by resultant conformational changes, but without massive interpolymer energy transfer and breakage of covalent bonds. (Kempner and collaborators)

Morphogenesis

Calliphorin. ^{14}C-phenylalanine injected into immature blowfly larvae was used to prepare high specific activity storage protein (calliphorin). The purified, tagged protein was injected into mature larvae, and various stages and adult organs examined for radioactivity. Of the injected dpm, 10% occurred in the empty puparium, and ~ 3% was oxidized during the 10 days of adult development. At 5 days of adult development calliphorin was the only labeled protein observed in the insect, but at adult emergence, and in the 48 and 96 hours adult virtually all proteins of the fly head, thorax and abdomen were now radioactive, as were all the adult organs. A major role of calliphorin, therefore, appears to be that of an amino acid depot, for the liberation, and prompt utilization, of amino acids involved in adult protein synthesis. (Levenbook)

Platelet aggregation. Platelet filament formation and aggregation is enhanced when the barometric pressure is reduced to 1/2 atmosphere. These effects are inhibited by piracetam (2-oxo-1 pyrolidine acetamide) at a concentration of 0.1 to 0.5 mM. These observations may explain the mechanism of acute mountain sickness and the apparent ability of piracetam to prevent this syndrome. (Murayama)

Sense of Rhythm

The basic problem of how photic signals are processed by the central nervous system so as to control the timing of reply signals was investigated in the fireflies Photinus pyralis of Maryland and P. concisus of Texas. By means of image-intensified videoscopy in the field and recording photometry in the laboratory it was shown in P. pyralis that flashes perceived between 4.5 and 6s after the male's flash (normal free run period 6s) evoke an immediate response (latency 400 ms). As a result, several neighbor males may become self-entrained into essentially synchronized flashing. P. concisus shows a comparable male-male triggering in both field and laboratory (latency 650 ms) but, unexpectedly, shows also an alternative response with the same latency as the free run flashing period (2.2s). P. concisus, therefore, can be entrained by a rhythmic series of artificial signals in much the same way as certain Oriental firefly species (and human beings), although over a narrower frequency range. Flash control timing thus shows unexpected complexity, raising questions about the validity of the view that there are two major types of timing response, one in which the timing cycle is terminated immediately, with the effector machinery being set in motion simultaneously, and the other, in which the timing cycle is immediately aborted and set back to its starting point, thus delaying response for a full timing cycle but making possible exact coincidence of two or more entrained rhythms. (Buck)

High Resolution Electron Microscopy

The goal of this project is to elucidate structural aspects of the assembly of protein subunits and other macromolecules into structures at the next higher level of biological organization - such as virus particles, systems of cytoskeletal filaments, and membranes. In addition to continued development of experimental resources, results have been achieved on three biological projects.

Crystals of Human IgG - Specificity of Protein Staining with Uranyl Acetate. Aiming in the long-term to solve crystal structures by electron optical methods, an essential first phase of this project has been an objective evaluation of the information content of such micrographs. Using the known crystal structure of the myeloma immunoglobulin Dob as model system, we have estimated the proportion of specific (positive) staining of charged amino-acid residues present in computer-optimized micrographs of "negatively stained" specimens. The outcome - a consistently small fraction (10-40%, and usually about 15%) of positive staining - is of considerable importance in that it establishes the contrast enhancement mechanism of negative staining as bulk exclusion of heavy metals by protein: in consequence, interpretation of structures derived from such micrographs may be made directly, and is not conditional upon prior knowledge of the three-dimensional distribution of charged residues. (Steven, Navia (LMB))

Virus Structures - Capsid Organization of Bacteriophage T7. We have investigated the surface shell of this spherical dsDNA - containing bacteriophage, analyzing polycapsids - tubular capsid analogs (mis)assembled on account of a lesion in a morphogenetic gene. Image processing methods have been used to define a hexagonal surface lattice of repeat 12.6 nm, and a hexamer of gp10 protein subunits as the repeating unit. Systematic analysis of the range of foldings assumed by this lattice implies form-determining specificity for the protein in terms of a preferential radius of curvature of 29 nm. By collating these results with earlier observations, we have developed a three-dimensional model of the capsid as an orthodox icosahedron of triangulation class T=7, comprising 60 hexamers and 12 pentamers of gp10. Significantly, these findings rule out the alternative of a 72-pentamer structure, such as has been recently proposed for the capsid of the structurally comparable polyoma virion (Steven, Trus (DCRT), Serwer (U. Texas))

Structure and Distribution of Mass in Fibroblastic Intermediate Filaments. Both to measure the efficiency and fidelity of the *in vitro* assembly reaction of IF from their constituent subunits, and to impose a firm constraint upon feasible models for the packing of subunits, we have analyzed fibroblastic IF from Chinese Hamster ovary cells both in the native state and after *in vitro* reassembly from purified subunits. Native specimens average 38 ± 4 kD/nm and also contain a minor population at 24 ± 4 kD/nm. Some of the latter type may represent contaminating microfilaments (actin polymers) but others clearly represent a polymorphic variant of decamin polymer, possibly an assembly intermediate. *In vitro* assembly is efficient and evidently faithful; the principal assembly product averages 37 ± 4 kD/nm; in addition, lesser amounts of species at 26 ± 4 kD/nm and 52 ± 5 kD/nm respectively are also present. Negatively stained specimens clearly demonstrate the organization of individual filaments to be based on bundles of fibrous 'protofilaments' aligned approximately parallel to the filament axis. (Steven, Steinert (DB:NCI)).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 27000-20 LPB

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

The Mechanism of Muscular Contraction

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. J. Podolsky	Chief, LPB	LPB NIADDK
OTHER:	L. C. Yu	Research Physicist	LPB NIADDK
	T. Arata	Visiting Fellow	LPB NIADDK
	B. Brenner	Visiting Fellow	LPB NIADDK
	T. Matsuda	Visiting Associate	LPB NIADDK

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Physical Biology

SECTION
Section on Cellular Physics

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 5.5	PROFESSIONAL: 3.5	OTHER: 2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES X☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The structural changes associated with the transition of muscle fibers from one physiological state to another have been examined by X-ray diffraction techniques, using radiation from conventional rotating anode generators and synchrotrons in conjunction with electronic, position sensitive, X-ray detectors. The rate constants associated with the turnover of actomyosin cross-bridges have been studied by analyzing the contraction kinetics of skinned muscle fibers under controlled chemical conditions. The mechanical properties of muscle fibers the rigor (ATP-free) state have been analyzed as a function of myofilament lattice spacing to obtain information about the geometry of the actomyosin cross-bridges.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 27001-08 LPB

PERIOD COVERED

October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Contractility and Excitability of Skeletal and Cardiac Muscle

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Mark Schoenberg	Medical Officer	LPB NIADDK
	Bernhard Brenner	Visiting Fellow	LPB NIADDK

COOPERATING UNITS (if any)

Evan Eisenberg, LC/NHLBI

LAB/BRANCH

Laboratory of Physical Biology

SECTION

Section on Cellular Physics

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, MD 20205

TOTAL MANYEARS: 1.3	PROFESSIONAL: 1.3	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

At $5^{\circ}C$, the stiffness of a relaxed muscle at low ionic strength scaled with the amount of overlap between the actin and myosin filaments. This suggests that this stiffness is due to attached cross-bridges and provides the first evidence that crossbridges can be attached in a relaxed muscle without producing active force. This in turn suggests that at low ionic strength, relaxation is caused by a mechanism other than the classic steric blocking mechanism.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM-27002-19 LPB

PERIOD COVERED October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Sense of Rhythm

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	John B. Buck	Research Physiologist	LPB-NIADDK
OTHER:	Mrs. Elisabeth M. Buck	Guest Worker	LPB-NIADDK

COOPERATING UNITS (if any) Dr. Frank E. Hanson, Dept. of Biology, Univ. of Maryland, Catonsville, Md.; Dr. James F. Case, Dept. Biological Sciences, Univ. of California, Santa Barbara, California.

LAB/BRANCH
Laboratory of Physical Biology

SECTION
Comparative Physiology Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland

TOTAL MANYEARS: 2	PROFESSIONAL: 1	OTHER: 1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The basic problem of how photic signals are processed by the central nervous system so as to control the timing of reply signals was investigated in the fireflies Photinus pyralis of Maryland and P. concisus of Texas. By means of image-intensified videoscopy in the field and recording photometry in the laboratory it was shown in P. pyralis that flashes perceived between 4.5 and 6s after the male's flash (normal free run period 6s) evoke an immediate response (latency 400 ms). As a result, several neighbor males may become self-entrained into essentially synchronized flashing. P. concisus shows a comparable male-male triggering in both field and laboratory (latency 650 ms) but, unexpectedly, shows also an alternative response with the same latency as the free run flashing period (2.2 s). P. concisus, therefore, can be entrained by a rhythmic series of artificial signals in much the same way as certain Oriental firefly species (and human beings), although over a narrower frequency range. Flash control timing thus shows unexpected complexity, raising questions about the previously accepted dichotomy between directly triggered and resettable pacemakers.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 27003-13 LPB

PERIOD COVERED October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

The Dynamic Properties of Cell Membranes and Related Systems

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Norman L. Gershfeld	Research Chemist	LPB-NIADDK

COOPERATING UNITS (if any) Kenneth M. Kent, CB, NHLBI; K. Pettigrew and C. Patlak, TSB, NIMH; Health Services Foundation, Livermore, California; Lionel Ginsberg, Middlesex Hospital Medical School, London, England.

LAB/BRANCH Laboratory of Physical Biology

SECTION Section on Comparative Physiology

INSTITUTE AND LOCATION NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.3	1.3	1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Permeability of lecithin surface bilayers.

The structure of lecithin bilayers that form spontaneously at the air-water surface has been examined by water permeability measurements; these have been achieved by studying the rate of evaporation of water in the presence of the bilayer. At the temperature of formation of dimyristoyl lecithin surface bilayers (28.9°C) the rate of evaporation of water was decreased by approximately 50% from that of a lipid-free water surface. Raising or lowering the temperature as little as 0.1°C increased the rate of water evaporation to the value for the lipid-free system. Thus, it is established that surface bilayers form in a very narrow temperature interval (0.1°C) and are a significant diffusion barrier to water. This phenomenon may have special significance with respect to the influence of temperature on membrane permeability, especially in the case of depolarizing potentials in excitable tissues.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 27004-12 LPB

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Physical Chemistry

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Melvin H. Gottlieb	Research Chemist	LPB-NIADDK

COOPERATING UNITS (if any)

Dr. Paul D. Smith, BEIB-DRS-NIH

LAB/BRANCH Laboratory of Physical Biology

SECTION Section on Comparative Physiology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Earlier work showed that (enzymatic) hydrolysis of inner leaflet phospholipid of the bilayer of the erythrocyte membrane increases the rate of the dissociation of cytoskeletal proteins which takes place at low ionic strength. It has now been established that this increased rate results from the presence in the membrane of hydrolysis products, the lysophospholipids. The effects of a number of other substances on the dissociation were examined in view of the possibilities (a) that the association in normally functioning cells depends on an appropriate concentration of some substance, and (b) that the widespread physiological effects of local anesthetics are due to modification of bilayer-cytoskeleton interactions. Only two of the substances examined, neither of them anesthetics, increased the dissociation rate of the cytoskeletal proteins. One is phosphoryl choline, which is structurally related to the polar groups of the lysophospholipids. The other is 2,3 diphosphoglycerate (DPG), which is normally present in red cells. Additional studies are necessary to determine if DPG affects bilayer-cytoskeletal association under physiological conditions.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM-27006-25 LPB

PERIOD COVERED October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Developmental biochemistry of insect metamorphosis.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	L. Levenbook	Research Chemist	LPB-NIADDK
OTHER:	A. Bauer	Research Physiologist	" "

COOPERATING UNITS (if any)

None

LAB/BRANCH Laboratory of Physical Biology

SECTION Comparative Physiology Section

INSTITUTE AND LOCATION

NIADDK/NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 2	PROFESSIONAL: 2	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Earlier experiments on the fate of blowfly storage protein (calliphorin) have been confirmed, and extended, using tagged calliphorin of higher specific activity than was previously available. It was demonstrated that virtually all the separated proteins of the adult head, thorax and abdomen contained radioactive phenylalanine and/or tyrosine derived from the labeled calliphorin. Significant fractions of the administered radioactivity remained in the pupal case, and was expired as $^{14}CO_2$. Two manuscripts were written, submitted, and accepted for publication; a third manuscript is in preparation, and an extensive review of insect storage proteins has been completed.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM-27007-20 LPB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Molecular Mechanism of Human Red Cell Sickling & Blood Coagulation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. Murayama	Research Chemist	LPB-NIADDK
OTHER:	None		

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Physical Biology

SECTION
Cellular Physics Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland

TOTAL MANYEARS: 0	PROFESSIONAL: 0	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Inactive

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM-27008-23 LPB

PERIOD COVERED

October 1, 1981 to September 20, 1982

TITLE OF PROJECT (80 characters or less)

Studies of metabolic activity in microorganisms

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. S. Kempner	Physicist	LPB-NIADDK
OTHER:	J. H. Miller	Chemist	LPB-NIADDK

COOPERATING UNITS (if any) Dr. M. Schotz, VA Hospital; Dr. S. Erickson, Stanford; Dr. Lefkowitz, Duke; Dr. R. Kaback, Hoffman-LaRoche; Dr. S. Paul, NIMH; Dr. P. Scolnick, NIADDK; Dr. R. J. Turner, NHLBI; Dr. M. Lowe, NIADDK.

LAB/BRANCH

Laboratory of Physical Biology

SECTION

Comparative Physiology Section

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The relationships between biochemically sequential reactions in polyenzyme complexes is being examined. Radiation inactivation is being used to analyze these systems and also the various membrane receptor systems is being used. Studies of the fundamental mechanisms of radiation damage to macromolecules are being conducted.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 27009-04 LPB

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Macromolecular Structure

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Alasdair C. Steven	Visiting Scientist	LPB NIADDK

COOPERATING UNITS (if any)
Other collaborators on specific projects are identified below, in the detailed accounts of these activities.

LAB/BRANCH
Laboratory of Physical Biology

SECTION
Section on Cellular Physics

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	1	1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Structural studies on biological macromolecules and macromolecular assemblies have been performed by high resolution electron microscopy complemented by optical diffraction and digital image processing. Micrographs of human immunoglobulin IgG crystals have been analyzed extensively to determine their structural fidelity. Compared to reference images derived from X-ray crystallography, the computer-optimized micrographs show excellent fidelity to 2 nm resolution and a predominantly non-specific distribution of the uranyl stain. The capsid structure of bacteriophage T7 has been solved by processing micrographs of polycapsids, tubular foldings of the viral surface lattice, thereby resolving hexamers of the major capsid protein, gp10. These results have been developed into subunit packing schemes for the tubes, as well as for the icosahedral wild-type capsid. Direct mass measurements of fibroblastic intermediate filaments have been effected by image analysis of scanning transmission electron micrographs: these findings have established the efficacy of the in vitro assembly reaction and imposed a stringent constraint on feasible geometries for the arrangement of fibrous subunits within these filaments.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM-27010-01 LPB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
ACUTE MOUNTAIN SICKNESS: Decompression Platelet Aggregation Cause, Platelet Inhibitors Prevent

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. Murayama	Research Chemist	LPB NIADDK

OTHER: None

COOPERATING UNITS (if any) Prof. Ray Henry and Dr. Robert M. Nalbandian, Wayne State University, Detroit, Michigan; Dr. Genesio Murano, Bureau of Biologics, Div. of Blood and Blood Products; K. K. Kamaroo, U.S. Naval Research Lab.

LAB/BRANCH
Laboratory of Physical Biology

SECTION
Cellular Physics Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	1	0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

When the barometric pressure is reduced to 380 mm Hg (1/2 atmosphere) I found that platelets aggregated independently of pO_2. By analogy with the vascular occlusion caused by sickled erythrocytes in sickle cell crisis the hypothesis is proposed that reduced atmospheric pressure (decompression) at high altitude causes platelet aggregation which partially occlude arterioles and causes acute mountain sickness (AMS). In both disabilities the damage is caused by hemostatic plugs adhering to the endothelial wall, plugs composed in the one instance of sickled erythrocytes, in the other of aggregated thrombocytes. Because I have found that piracetam (2-oxo-1 pyrolidine acetamide) at concentration of 0.1 to 0.5 mM prevents thrombocyte aggregation, I suggest that the drug might prevent or alleviate AMS. I also found that piracetam renders fibrin polymer more soluble. I predicted and found that NaBr (40 mM) prevented platelet aggregation. It is interesting to note that Dr. Ricardo Manrique, University of São Paulo, Brazil found that in a clinical trial piracetam and pentoxofillin (another anti-platelet drug) prevented AMS.

ANNUAL REPORT OF THE LABORATORY OF CHEMICAL PHYSICS

This report is dedicated to Giovanni Lipari whose tragic death occurred just as the report was being written.

The Laboratory of Chemical Physics consists of a group of scientists and support staff engaged in research on the application of physical methods to problems in molecular and cellular processes. These problems range from questions concerning the fundamentals of the electronic and atomic properties and architecture of molecules, to the way in which these properties are reflected in the behavior of both normal and abnormal biological processes. As such it is a microcosm of the NIH research effort as a whole, with the exception that no direct medical intervention with humans is involved. The programs of the scientific staff as they are reflected in their substantial accomplishments this year will be individually traced. A close reading will demonstrate the common interest in the way fine details of molecular and electronic structure determine both the static and dynamic properties of molecules ranging in size from the few atoms of small organic molecules like cyclohexadienes to the millions of atoms in DNA or in the complexes which form cell membranes, and the shared interests in the use of advanced electromagnetic techniques with frequencies ranging from long radiofrequency waves used in electron and nuclear magnetic resonances to the ultra-high frequencies associated with the so-called vacuum ultraviolet spectrum.

The following summary is in the order of the intramural research project number and is limited to the principal accomplishments associated with the projects. In each case additional significant and important results were obtained; these will be found in the reports of the individual principal investigators.

Becker: The high concentration of very expensive nuclear magnetic resonance (NMR) equipment with its associated computers has enabled the laboratory to act as host to investigators from other laboratories at NIH as well as from outside NIH. Descriptions of the work of some of these investigators (Cohen/NICHD; Torchia/NIDR) is therefore found elsewhere in the annual reports.

Inubushi has made extensive NMR studies of several heme proteins and heme enzymes. Among the most interesting results are those for hemoglobin in which the iron atoms are replaced by cobalt in either the α or β hemes. Carbon monoxyhemoglobin is then prepared, in which it is known that the CO attaches only to the iron, not the cobalt. (Such partially liganded molecules serve as models for partially liganded oxyhemoglobin.) By following the proton chemical shift of the NH in the proximal histidine F8, which is directly bound to the paramagnetic Fe or Co, Inubushi has shown that complexation of CO in the β heme causes a structural change in the α heme such that the F8 histidine is brought significantly closer to the cobalt. By contrast, complexation of CO in the α heme causes virtually no change in the histidine resonance of the β heme, hence presumably little or no structural change in this subunit.

Baltzer and Becker have completed a study by ^{17}O NMR of the solvation of certain oxy-anions. Nuclear relaxation of ^{17}O provides a sensitive probe into the size of the moiety that tumbles in solution--i.e., the anion itself plus associated solvent molecules. For the anions of benzoic acid and p-nitrophenol in mixed solvents of varying proportions of methanol and dimethylsulfoxide, it has been shown that even relatively small mole fractions of DMSO break the strong methanol-anion hydrogen bond and lead to partial desolvation.

Levin: This project utilizes vibrational Raman and infrared spectroscopy to probe and assess the dynamical and conformational properties associated with lipid-lipid and lipid-protein interactions in various membrane assemblies. Huang and Levin examined the phase transition characteristics for the members of an homologous series of saturated, symmetrical chain phosphatidylcholine bilayer dispersions for even carbon chain length systems extending from diC(10) to diC(22). Raman spectral changes were correlated with thermodynamic parameters determined by calorimetry. Huang and Levin have also characterized the previously ignored lamellar to micellar transitions of 1-palmitoyl and 1-stearoyl lysophosphatidylcholines. These Raman studies suggest that the acyl chains of opposing leaflets are nearly completely interdigitated in the lamellar structures. Transitions of micellar structures are observed which are sharper than the gel↔liquid crystalline transitions of their diacyl derivatives. Reverse micellar to lamellar transitions were observed by Raman techniques to occur quite slowly.

In order to monitor perturbations to membrane assemblies at the interfacial region of the bilayer, Mushayakarara and Levin investigated the behavior of the carbonyl spectral region for a variety of lipid systems. Spectra were analyzed in terms of lipid reorganizations involving selectively either of the two inequivalent acyl chain species.

Virtually no information exists concerning protein or polypeptide structure within membrane bilayers. Lavialle, Adams and Levin examined the infrared spectra of melittin, a 26 amino acid polypeptide, and its hydrophobic (membrane intrinsic) and hydrophilic (membrane extrinsic) fragments both in solvents and in lipid bilayer dispersions in order to characterize the various conformational changes available to the polypeptide. The α-helical conformation of intact melittin in membrane bilayer dispersions remains unchanged as the bilayer passes from the gel to liquid crystalline state and through the melting of the polypeptide's immobilized boundary lipids. Lapides and Levin used both Raman and calorimetric techniques to characterize the bilayer to hexagonal II (a non-bilayer form for the membrane) phase transition for dioleoyl- and 1-palmitoyl-2-oleoyl-phosphatidylethanolamines. In addition to elucidating the hexagonal II states, several Raman spectral parameters proved sensitive to additional polymorphic structures.

Adams and Levin applied infrared techniques to assess the effects of the extrinsic basic myelin protein on the structural and reorganizational properties of the interdigitated bilayer assemblies of dipalmitoyl phosphatidylglycerol.

A considerable instrumental advance has been made by coupling a Raman spectrograph system to a state-of-the-art multianode, multichannel diode array detector. Up to three orders of magnitude in time have been saved in the acquisition of the weak Raman signals required from membrane assemblies for the recording of spectra and the determination of temperature profiles.

Sollner (Scientist Emeritus): Finished an advanced draft of his planned "History of the Physical Chemistry of Membranes" which covers the period before the creation of the classical theory of electrolytic solutions, just before 1890.

Weiss (Scientist Emeritus): Collaborative research with Dr. James Cook (University of Wisconsin) on the general synthesis of polycyclic five-membered ring systems has continued and the unusual reactivity of these systems has been explained. In another joint project with Highet (NHLBI/LC) and a group of scientists at the FCRC, Frederick, Md., the structure and stereochemistry of the chrysomycins A and B have been completely elucidated. These antibiotics (perhaps only A) show significant antileukemic action in mice and are well tolerated in intraperitoneal application.

Ziffer and Kasai have continued their studies of microbially mediated reactions. Their recent studies have explored the scope and limitations of a rule proposed earlier, to predict the absolute stereochemistry of alkylarylcarbinols formed from the hydrolysis of the corresponding acetates by *Rhizopus nigricans*. A number of new compounds have been prepared to determine the effect of more distant substituents on the stereochemical course of these hydrolyses. A study of the relationship between elution order from a chiral HPLC column and the absolute stereochemistry of alkylarylcarbinols and their acetates has been completed. Although some exceptions were noted, an enantiomer's elution order can be used to assign tentatively the absolute stereochemistry of these compounds.

Charney: The structure and properties of DNA and model polymeric polynucleotides have been studied using the technique of electric-field induced orientation in solution described in previous reports. It has now been demonstrated that a left-handed form of the DNA-like polynucleotide, poly (dGm^{5}dC) has precisely the same structure in solution as it does in solid fibers examined by x-ray diffraction. These results and those of a similar study of poly (dAdT) now underway are expected to help solve the paradox which now exists in the structural modeling of DNA in chromatin. In a separate project with R. Charney (Yale University) and Feldman (DCRT), a model of chromatin in which DNA has a tertiary structural superhelical coil has been proposed. A number of other studies of DNA and chromatin structure and properties are underway. These studies are aimed in particular at understanding the high density of packing of DNA in biological structures. For example Rau with Lee and Parsegian (PSL/DCRT) have been characterizing DNA-DNA strand interactions by determining their separation (using x-ray diffraction techniques) as a function of the osmotic pressure exerted on them by the solvent. With McGhee and Felsenfeld (LMB/NIADDK) and Allan and Gould (Kings College, London), Rau has also found that chromatin in which the small core histone "tails" have been removed with trypsin cannot fold back into the regular compact chromatin structure. The thermodynamic driving forces for DNA conformational transitions such as those which may occur as preludes to, or during, transcription and replication, are being studied with Szu. The first result shows that the enthalpy change accompanying transitions of poly (dGm^{5}dC) and poly (dGdC) to the left-handed Z structures are of the order of 30 and 200 kcal/mol or more on a phosphate basis respectively.

Kon: The novel spin label method for studying red blood cell deformability described in the previous annual report has been applied to an investigation of the effects of various drugs and reagents on the cell deformability. One of the principal findings is that, contrary to conventional wisdom, the recent theoretical prediction by Brailsford *et al*. (J. Theor. Biol. *86*, 531 (1980)) that red blood cell stomatocyte (cup shaped cell) formation results from a reduction in the cell membrane bending resistance appears to be correct. Using the electron spin resonance technique, Kon and Noji have shown that an increase in membrane fluidity, which is a concomitant of a reduction in membrane bending resistance, is associated with four different techniques for inducing stomatocyte formation.

McDiarmid: Spectroscopic investigations of the excited electronic states of prototype polyatomic molecules are motivated by the realization that it is necessary to understand the energetics of the states of small molecules before it is possible to understand those of larger molecules of biological interest. The work uses the most advanced "state of the art" equipment and techniques with variable frequency dye lasers, electron impact spectroscopy (in cooperation with J. Doering at Johns Hopkins University) and multiphoton ionization (MPI) spectroscopy. Typical examples of the new results this year are (1) the demonstration that the supposed low lying symmetry forbidden transition of all *trans* polyenes is really an allowed transition in the partially *s-cis* isomerized moieties of the corresponding polyenes. A long standing theoretical question about the energy levels of polyenes is thus resolved; and (2) the extension of the multiphoton ionization spectroscopic method to the observation and analysis of methyl substituted dienes.

Eaton: The principal objective of this investigation continues to be the elucidation of the molecular mechanism of hemoglobin S gelation in order to provide a rationale for the development of theropeutic agents for sickle cell disease. In cooperation with Hofrichter, Coletta, Bunn (Harvard Medical School) and Ferrone (Drexel University) the following specific advances have been made this year: (1) the mathematical treatment of the kinetics of hemoglobin S polymerization has been improved to allow a critical evaluation of the previously developed double nucleation model; (2) a study of other sickle cell syndromes has begun and the first data shows that the delay time (time before critical rapid polymerization of the blood occurs) in SC disease is significantly shorter than in AS trait and follows the differences found in clinical manifestation and (3) thermodynamic and kinetic experimental data indicate that the copolymerization tendencies of hemoglobin C and A at the same composition are identical. (4) kinetic studies on single cells has shown that the mechanism of hemoglobin S polymerization is the same as in purified solutions.

Eaton: Molecular dynamics (the change of atomic coordinates of a molecule with time) computer simulations of large molecules utilizing programs developed by Michael Levitt (Weizmann Institute, Rehovot, Israel) have been modified and adapted by Eric Henry for the laboratory PDP-11/70 computer and the Laboratory of Molecular Biology VAX-11/780 computer and are now being utilized to examine the structural response of a hemoglobin chain to the removal of an oxygen or carbon monoxide molecule from the heme group. It has already been learned that the motion of the iron atom out of the heme plane may occur on a time scale as small as 10^{-13} sec and that the oxygen or carbon monoxide ligand may diffuse to the periphery of the protein in 10^{-11} sec or less.

Another aspect of this project, the use of a transient laser spectrometer for extremely rapid measurements of optical absorption spectra and kinetics of hemoglobin conformational kinetics is discussed in H. J. Hofrichter's project description.

Hagins and Yoshikami: This joint project of two principal investigators involves studies of the visual processes by optical and electrical measurements in live rod cells. To this has now been added the investigation of the cellular mechanism of visual transduction by new experimental techniques of electron beam microanalysis and radioautography together with standard biochemical methods. It has been shown that retinal rod disk membranes store calcium ions in darkness by an energy dependent and cyclic GMP dependent uptake mechanism. The oft questioned nature of the association of Ca^{++} with the visual process now seems clear. Light quickly releases at least 10,000 calcium ions per absorbed light quantum (George, Foster, Hagins). The resulting elevation in cytoplasmic Ca^{++} activity transiently alters the dark current produced by the cells by polarizing them to produce the fast neural event of visual excitation. Ca^{++} in the cytoplasm of retinal rods is controlled by an extrusion mechanism that depends on Na:Ca exchange and is driven by the thermodynamic gradient of Na^{+} across the plasma membrane. The rate of ejection of Ca^{++} strongly affects the termination of light responses in retinal rods (Yoshikami).

Sharpless, Jennings and Adams: Investigation of the structure of retinaldehyde isomers and of the binding site structure of aldose reductase by molecular modeling via computerized energy minimization has continued. In the case of the latter, the results permit an analysis of the mechanism of inhibition of its action by flavonoids and related compounds. The theoretical computations give the shape and dimensions of the aldose reductase site, the electron availability and lipophilic and hydrogen bonding effects. The retinal calculations of seventeen different isomers show that a number of physical parameters separate the forms assumed to be involved in the visual process from the others.

Jennings: With the aid of the Computer Systems Laboratory, DCRT, the laboratory computer facility serving users in LCP and LMB in Building 2 has been converted from a ten year old Honeywell System to a new and more diversified system based on a Digital Equipment PDP 11/70. Extensive programming and re-engineering of terminal hardware has been virtually completed (Jennings, et al.).

Hofrichter: The nanosecond transient spectrometer developed in this laboratory has been further improved and used to systematically examine the kinetics of photolysis-induced reactions of several heme-protein complexes. Sommer and Henry have collected and analyzed data from the spectrometer for the carbon monoxide complexes of myoglobin and hemoglobin. In addition, preliminary data have been collected on several other systems. The results for hemoglobin reveal a sequence of ligand binding and spectral events which are interpreted as (1) geminate rebinding of protein-bound ligand which has been dissociated from the heme (10^7 sec^{-1}); (2) escape of the ligand from the protein (10^7 sec^{-1}); (3) tertiary structural relaxation (10^6 sec^{-1}); (4) quaternary structural relaxation ($2x10^5$ sec^{-1}); and (5) recombination of ligand from solution to the mixture of R ($6x10^6 M^{-1}$ sec^{-1}) and T quaternary states ($1x10^5 M^{-1}$ sec^{-1}). In myoglobin only three processes are observed, the geminate recombination which is considerably slower than in hemoglobin ($5x10^5$ sec^{-1}), the escape which occurs at a comparable rate to hemoglobin and a single bimolecular recombination process. These results provide an outline in which to examine the ligand binding reaction to heme proteins in terms of elementary steps. The present data characterize the path for ligand dissociation and provide overall rates for the rebinding process.

Studies of the kinetics of sickle cell hemoglobin polymerization have been focused on measuring the rate of homogeneous nucleation is a process by which individual polymers are initiated by sequential addition of monomers. This process has been proposed as the rate-controlling step in the polymerization of hemoglobin S based on the extreme dependence of the polymerization kinetics on temperature, concentration and solubility. Experiments have been carried out on very small volumes where a single nucleation event controls the polymerization of all the hemoglobin in the observed volume.

The distribution of times observed for the nucleation step in these experiments provide strong support for the double nucleation mechanism for sickle cell hemoglobin polymerization. They also permit quantitative characterization of the homogeneous nucleation reaction which is not possible from kinetic experiments on bulk samples. They further show: (a) unambiguous measurements of the homogeneous nucleation rate are possible from the distribution of delay times and that under a single set of conditions, (b) the probability of homogeneous nucleation is constant (independent of time) until polymerization takes place.

Gansow: Gansow has made spectacular progress in producing radio-labeled antibodies to tumor cells for use in cancer diagnosis and therapy. This work is being done in collaboration with Strand and Scheinberg of Johns Hopkins Medical School, who have developed monoclonal antibodies with high specificity to glycoproteins found in the spleens of mice that have been given virus-induced leukemia. With γ-emitting ^{111}In attached to the antibody in the form of appropriate chelates, excellent imaging of the spleen with little radioactive background was achieved. For therapy α-emitting isotopes offer the most promise because of the high, localized destructive power of α particles. Much of the effort during the last year has gone into developing a generator for ^{212}Pb and ^{212}Bi and working out the complex chemistry needed to attach such isotopes to antibodies. A new bifunctional chelate, a N-hydroxysuccinimide ester derivative of benzyl EDTA has been prepared, which permits rapid incorporation of the isotope, attachment to the antibody and purification of the complex.

Szabo: Theoretical approaches have been developed to extract information concerning the nature of internal motions in a variety of biopolymers from nuclear magnetic relaxation and time-resolved fluorescence depolarization experiments. Together with Lipari the unique information content of NMR relaxation experiments on macromolecules has been established and a model-free approach to obtain that information has been proposed and extensively applied. A theory required to analyze solid state spin-lattice relaxation times has been developed. Contact has been made between molecular dynamics simulations and fluorescence depolarization of tyrosines in proteins. In addition, the role of diffusion in ligand binding to macromolecules and cell-bound receptors, where there is competition between reactive sites, has been analyzed. Finally, together with Shoup the theory of the chronoamperometric current to microelectrodes has been worked out.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-AM-29000-10-LCP

PERIOD COVERED

October 1, 1981, through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Nuclear magnetic resonance: New methods and molecular structure determination

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI :	Edwin D. Becker	Research Chemist	A LCP
OTHER:	Rolf G. Tschudin	Electronics Engineer	A LCP
	Toshiro Inubushi	Visiting Fellow	A LCP
	Lars H. Baltzer	Guest Worker	A LCP

COOPERATING UNITS (if any)

C. L. Khetrapal, Bangalore NMR Facility, Bangalore, India
T. Yonetani, University of Pennsylvania
I. Baltzer, Walter Reed Army Institute of Research

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Laboratory of Chemical Physics

SECTION

Section on Nuclear Magnetic Resonance

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 3	PROFESSIONAL: 3	OTHER:

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Iron-cobalt hybrid hemoglobins have been used to show via proton NMR studies that complexation of CO results in a structural change localized near the hemes of the α subunits, not the β subunits.

An improved method to reduce the interference from solvent H_2O signals in proton NMR studies of paramagnetic proteins--"super WEFT"--has been developed and tested.

The extent of solvation on the anions of benzoic acid and p-nitrophenol in mixed solvents of methanol and dimethylsulfoxide has been elucidated by measurements of ^{17}O NMR relaxation rates.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-AM-29001-10-LCP

PERIOD COVERED

October 1, 1981, through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Molecular dynamics and vibrational characteristics of membrane assemblies

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI :	Ira W. Levin	Research Chemist	A LCP
OTHER:	Ernest Mushayakarara	Visiting Fellow	A LCP
	Ching-hsien Huang	IPA	A LCP

COOPERATING UNITS (if any)

R. Adams, LCP-NIADDK
J. Boggs, The Hospital for Sick Children, Toronto, Canada
H. Mantsch, National Research Council, Canada

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Laboratory of Chemical Physics

SECTION

Section on Molecular Biophysics

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

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4	3	1

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Vibrational Raman and infrared spectroscopic transitions are used to probe the dynamical and conformational properties associated with lipid-lipid and lipid-protein interactions in membrane systems. For example, the phase transition characteristics for the members of an homologous series of saturated, symmetrical phosphatidylcholine bilayer dispersions were investigated by Raman spectroscopy for even carbon chain length systems extending from C(10) to C(22). A Raman spectral parameter ΔI_R, which represents changes in a specific C-H stretching mode intensity ratio across the gel to liquid crystalline phase transition T_m, is correlated with ΔS, the entropy change at T_m determined by calorimetry.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-AM-29002-09-LCP

PERIOD COVERED
October 1, 1981, through September 30, 1982

TITLE OF PROJECT (80 characters or less)
Chemistry and biosynthesis of natural compounds, and instrumental methods used in their study

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Ulrich Weiss	Research Chemist (Scientist Emeritus)	A LCP

COOPERATING UNITS (if any)
James M. Cook, Department of Chemistry, University of Wisconsin-Milwaukee
Kenneth L. Kirk, NIADDK-LC; Robert J. Highet, NHLBI-LC
R. J. White, K. M. Byrne and T. T. Wei, Chemotherapy Fermentation Program, FCRC
Surendra P. Bhatnagar and B. B. Singh, Reckitt & Colman of India, Ltd., Bangalore

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Laboratory of Chemical Physics

SECTION
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INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	1	

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In cooperative with Dr. Cook and his pre- and postdoctoral coworkers, our general synthesis of di- and polycyclic ring systems composed of five-membered rings has been studied. Our earlier hypothetical interpretation of the reaction sequence involved in the formation of these compounds has been proved experimentally. The unusual reactivity of some of these systems has been explained, and one of them has been utilized for a new synthesis of the sesquiterpene modhephene.

The complete structure and stereochemistry of the antibiotics chrysomycin A and B have been elucidated, in cooperation with Dr. Highet and the group at FCRC. The antibiotic (mixture of 86% A and 14% B) has good antileukemic action on intraperitoneal administration to rats, and is tolerated in high doses.

It has been established in cooperation with Dr. Kirk that the chloromorphine obtained from morphine in dilute HCl with potassium iodate is indeed 1-chloromorphine, since it differs from authentic 2-chloromorphine, the alternative product which would actually have been expected on general principles. A plausible explanation for the anomalous formation of 1-chloromorphine has been given.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. D ARTMENT OF HEALTH AN HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-AM-29005-08-LCP

PERIOD COVERED
October 1, 1981, through September 30, 1982

TITLE OF PROJECT (80 characters or less)
Asymmetric synthesis; structure, stereochemistry and NMR

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI :	Herman Ziffer	Research Chemist	A LCP
OTHER:	Masaji Kasai	Visiting Associate	A LCP

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Chemical Physics

SECTION
Section on Molecular Biophysics

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	2	

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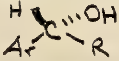
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER
☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The relation between the absolute stereochemistry of a series of acyclic alkylarylcarbinols and three groups of benzocycloalkenols (1-indanols, 1-tetralols and seven- and eight-membered benzocycloalken-3-ols) and their elution order from a chiral HPLC column ("Pirkle") has shown that in the acyclic and seven- and eight-membered benzocycloalken-3-ols the enantiomers with an 

configuration are preferentially retained by the column. While for 2-substituted 1-indanols and 1-tetralols the antipode is preferentially retained. In order to obtain these correlations it was necessary to prepare approximately fifty alcohols, enriched in one enantiomer whose configuration was either previously known or had to be established. These chiral alcohols were prepared using the microbial methods we had previously developed or are currently developing. Our correlations and the use of a chiral HPLC column permit one to characterize and tentatively assign the absolute stereochemistry of nanogram quantities of related aromatic alcohols.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-AM-29006-12-LCP

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
The relationship between the nuclear and electronic configuration of molecules and their spectroscopic and dynamic properties.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Elliot Charney

OTHER:	Donald C. Rau	Senior Staff Fellow	A:LCP
	Shousun Szu	Senior Staff Fellow	A:LCP
	Rodney L. Harrington	Guest Worker	A:LCP/IPA- Univ. of Nevada

Ruth Charney - Yal University
Holly Ho Chen - Univ. of Virginia
Richard Feldmann - DCRT:ECB
B.K. Lee - DCRT:PSL
V. A. Parsegian - DCRT:PSL

M. Behe - A:LMB
J.D. McGhee - A:LMB
G. Felsenfeld - A:LMB
J. Allan - Kings College, London
H. Gould - Kings College, London

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Chemical Physics

SECTION
Spectroscopy and Structure

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.5	3.5	2

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A. Macromolecular structure, dynamics and polyelectrolyte properties of large biological polymers, in particular, polynucleotides and nucleic acids are being studies by electric-field induced dichroism and birefringence methods. Theoretical and computational methods supplement the experimental work.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-AM-29007-11-LCP

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Structure and interaction of biomolecules

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Hideo Kon	Research Chemist	A:LCP
OTHER:	Sumihare Noji	Visiting Fellow	A:LCP

COOPERATING UNITS (if any)

T. Takahashi (American Red Cross)

LAB/BRANCH
Laboratory of Chemical Physics

SECTION
Spectroscopy and Structure

INSTITUTE AND LOCATION
NIH, NIADDK, Bethesda, Maryland 20205

TOTAL MANYEARS: 2	PROFESSIONAL: 2	OTHER:

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We have applied our spin label esr method to study the effect of drugs on the human red blood cell deformability under a steady shear flow as well as with regard to the deformation recovery time on cessation of the flow.

In a related area, the change of the membrane fluidity in the red blood cell was measured to investigate the mode of interaction of stomatocytogenic drugs and agents with the red cell membranes.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-AM-29008-11-LCP

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Electronic and molecular structural investigations.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Ruth McDiarmid	Research Chemist	A:LCP
Other:	Aleksandar Sabljic	Visiting Fellow	A:LCP
	Larry Schuette	Biological Aid	A:LCP

COOPERATING UNITS (if any)

John Doering - Johns Hopkins University

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Laboratory of Chemical Physics

SECTION
Spectroscopy and Structure

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 2.25	PROFESSIONAL: 2	OTHER: .25

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

1. The electron impact spectra of acetone and acetone-d_6 were measured and interpreted. The anomalously weak 7.4eV (59 700 cm^{-1}) transition was demonstrated to arise from the forbidden $X \rightarrow 3p(^1A_2)$ Rydberg Transition.

2. A resonant multiphoton ionization investigation of 1,4-cyclohexadiene confirmed the electron impact spectroscopic assignment of one optically allowed and one optically forbidden p-Rydberg transition, thus confirmed the theoretical model of thorough bond interactions.

3. Through interpretation of the electronic spectra of a large number of conjugated polyenes, the supposedly low lying, forbidden (A_g) valence transition of all trans-polyenes was shown to be an allowed transition in the partially s-isomerized corresponding polyene.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-AM-29009-09-LCP

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Studies on Sickle cell disease

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	William A. Eaton	Medical Director	A:LCP
OTHER:	James Hofrichter	Research Chemist	A:LCP
	Massimo Coletta	Guest Worker	A:LCP
	H. Franklin Bunn	Fogarty Scholar in Residence	

COOPERATING UNITS (if any)

Frank A. Ferrone	Assistant Professor	Department of Physics Drexel University

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Laboratory of Chemical Physics

SECTION
Macromolecular Biophysics

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	1	

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The thermodynamics and kinetics of hemoglobin S polymerization are being investigated in order to elucidate the molecular mechanism for this process. The results of these studies provide the rationale for developing agents that can be used in the treatment of patients with sickle cell disease. A method to measure the distribution of delay times for intracellular polymerization is being developed as an assay technique to test the efficacy of potential therapeutic agents in inhibiting intracellular polymerization of hemoglobin S.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-AM-29010-10-LCP

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Conformation and electronic structure of biological molecules

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	William A. Eaton	Medical Director	A:LCP
OTHER:	James Hofrichter	Research Chemist	A:LCP
	Eric Henry	Staff Fellow	A:LCP
	Joseph Sommer	Staff Fellow	A:LCP

COOPERATING UNITS (if any)

Michael Levitt	Associate Professor	Department of Chemical Physics The Weizmann Institute Israel

LAB/BRANCH
Laboratory of Chemical Physics

SECTION
Macromolecular Biophysics

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A transient laser spectrometer has been developed for measurements of optical absorption spectra and kinetics of hemoglobin conformational changes and ligand rebinding following photolysis of the carbon monoxide complex. Molecular dynamics calculations are being carried to simulate the heme structural changes and the ligand motion following photolysis.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-AM-29011-11-LCP

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)
The Physics and Chemistry of Photoreception

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	William A. Hagins	Medical Officer	A LCP
OTHER:	S. Yoshikami	Research Biologist	A LCP
	F. M. Hagins	Guest Worker	A LCP
	J. S. George	Biological Laboratory Technician	A LCP
	M. Foster	Guest Worker 1 July 1980-	(IPA)
	P. Rashidian	Guest Worker	A LCP
	H-C. Huang	Guest Worker	A LCP
	B. Trus	Research Chemist	CR CSL

COOPERATING UNITS (if any)

D. H. Blackburn	National Bureau of Standards Gaithersburg, Maryland

LAB/BRANCH
Laboratory of Chemical Physics

SECTION
Section on Membrane Biophysics

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

An investigation of the mechanism of visual excitation in vertebrates.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-AM-29012-12-LCP

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

The influence of molecular structure on chemical and biological properties

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Norman E. Sharpless	Research Chemist	A:LCP
OTHER:	Ralph G. Adams	Research Physicist	A:LCP
	William H. Jennings	Research Physicist	A:LCP

COOPERATING UNITS (if any)
Peter F. Kador, EI, LVR, NIH; Che-Hung Lee, C, LMC, NIH; C. H. L. Kennard, Reader in Chemistry, University of Queensland, Brisbane, Australia; and Steven H. Bertz, Bell Laboratories

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SECTION
Section on Membrane Biophysics

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Energy minimization calculations and quantum mechanical computations on compounds of biological and pharmacological interest continue to give insight into and explanations of their modes of behavior, resulting in clues to their pharmacophores.

The inhibition of the enzyme, aldose reductase by flavonoids is under investigation utilizing QSAR techniques as well as energy minimization, quantum mechanics and stereochemical considerations. A model for the interaction of a wide range of types of compounds with aldose reductase has been proposed, and the common features of the inhibitors obtained. The necessary critical molecular distances required have been estimated.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-AM-29014-22-LCP

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Effects of environmental stress (hypoxia, exercise, smoke) on animals

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Paul D. Altland	Guest Worker (Scientist Emeritus)	A LCP

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Chemical Physics

SECTION
Section on Physiology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project has been discontinued due to the retirement of Dr. Altland on August 21, 1981.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-AM-29015-11-LCP

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Digital computer facilities for LCP and LMB

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	William H. Jennings, Jr.	Research Physicist	A:LCP

COOPERATING UNITS (if any)
Computer Systems Laboratory, DCRT: Arthur R. Schultz, Jr., John I. Powell, Eugene O'Bryan, Ronald Fico

LAB/BRANCH
Laboratory of Chemical Physics

SECTION
Section on Membrane Biophysics

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 1	PROFESSIONAL: 1	OTHER:

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The laboratory computer facility serving LCP and LMB was in routine operation during the reporting period. This local network connects ten laboratory instruments with dedicated microcomputer systems to a large capacity host computer.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-AM-29016-07-LCP

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Macromolecular Dynamics and Assembly Reactions

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	James Hofrichter	Research Chemist	A:LCO
OTHERS:	William A. Eaton	Medical Director	A:LCP
	Eric Henry	Staff Fellow	A:LCP
	Joseph Sommer	Staff Fellow	A:LCP

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Chemical Physics

SECTION
Spectroscopy and Structure

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The transient spectrometer (see below) has been used to systematically examine the kinetics of photolysis-induced reactions of several heme-protein complexes. Complete data have been collected and analyzed for the carbon monoxide complexes of myoglobin and hemoglobin. In addition, preliminary data have been collected on several other systems. The results for hemoglobin reveal a sequence of ligand binding and spectral events which are tenatively interpreted as (1) rebinding of protein-bound ligand which has been dissociated from the heme (10^{7} sec^{-1}); (2) escape of ligand from the protein (10^{7} sec); (3) tertiary structural relaxation (10^{6} sec); (4) quaternary structural relaxation (2x10^{5} sec); and (5) recombination of ligand from solution to the mixture of R (6x10^{6} M sec) and T quaternary states (10^{5} sec). These results provide an outline in which to examine the ligand binding reaction to heme proteins in terms of elementary steps. The present data characterize the path for ligand dissociation and provide overall rates for the rebinding processes.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-AM-29017-03-LCP

PERIOD COVERED
October 1, 1981, through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Spectroscopic investigation of membrane lipids and models

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI :	Ralph G. Adams	Research Physicist	A LCP
OTHER:	Ira W. Levin	Research Chemist	A LCP

COOPERATING UNITS (if any)
J. Boggs, The Hospital for Sick Children, Toronto, Canada
Paul Smith and Horace Cascio, BEIB

LAB/BRANCH
Laboratory of Chemical Physics

SECTION
Section on Molecular Biophysics

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Infrared and Raman spectroscopic studies have been used to characterize conformational changes in the gel and liquid crystalline forms of simple model membrane systems.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-AM-29018-03-LCP

PERIOD COVERED
October 1, 1981, through September 30, 1982

TITLE OF PROJECT (80 characters or less)
The development of radiocryptate assisted imaging methods

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Otto A. Gansow	IPA	A LCP

COOPERATING UNITS (if any)
Dr. Mette Strand, Johns Hopkins Medical Institutions
Dr. David Scheinberg, Johns Hopkins Medical Institutions

LAB/BRANCH
Laboratory of Chemical Physics

SECTION
Section on Nuclear Magnetic Resonance

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Tumor-associated monoclonal antibodies are potential therapeutic agents as selective carriers of cytotoxic agents to malignant cells. We are testing this hypothesis in two animal model systems: a tumor virus induced leukemia of mice and human tumor xenographs in nude athymic mice.

The various cytocidal agents being employed are radioisotopes. Their relative therapeutic efficacy when conjugated to antibodies is being assayed and compared to that of monoclonal antibodies alone. The isotopes to be employed include the highly tumoricidal α-emitting parent radioisotopes ^{212}Pb or ^{212}Bi. The syntheses of different chelates and radiochemical separations required for these objectives are devised and reduced to clinical practice. Results from isotopic therapy being compared with those obtained by us of antibody conjugated toxins or drugs with respect to tumor growth, regression or cure.

These studies provide for human medicine a basis for design of rational therapy of malignancies by selectively targeting cytocidal agents to tumors as well as metastases.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-AM-29019-02-LCP

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Theoretical studies on the dynamic aspects of macromolecular function

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Attila Szabo	Visiting Scientist	A:LCP
OTHER:	Giovanni Lipari	Guest Worker	A:LCP
	David Shoup	Guest Worker	A:LCP

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Chemical Physics

SECTION
Macromolecular Biophysics

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

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3	3	

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Theoretical approaches are being developed to extract information concerning the nature of internal motions in a variety of biopolymers from nuclear magnetic relaxation and time-resolved fluorescence depolarization experiments. The unique information content of NMR relaxation experiments on macromolecules has been established and a model-free approach to obtain that information has been proposed and extensively applied. A theory required to analyze solid state spin-lattice relaxation times has been developed. Contact has been made between molecular dynamics simulations and fluorescence depolarization of tyrosines in proteins. In addition, the role of diffusion in ligand binding to macromolecules and cell-bound receptors, where there is competition between reactive sites, has been analyzed. Finally, the theory of the chronoamperometric current to microelectrodes has been worked out.

PHS-6040
(Rev. 2-81)

ANNUAL REPORT OF THE LABORATORY OF BIOORGANIC CHEMISTRY

SECTION ON OXIDATION MECHANISMS

Enzymatic Oxidation of Drugs to Toxic and Carcinogenic Metabolism

Previous annual reports from this Section have described a systematic approach to the study of the cytotoxicity, mutagenicity, and carcinogenicity of several polycyclic aromatic hydrocarbons. Briefly these studies have consisted of i) synthesis of as many known and potential oxidative metabolites as was possible, ii) study of the metabolism of the hydrocarbons with these authentic standards in hand, iii) testing these compounds for cytotoxic and mutagenic activity with bacterial and mammalian cells both in the presence and in the absence of added drug metabolizing systems such as cytochrome P-450 and epoxide hydrolase, and iv) evaluation of the carcinogenicity of these synthetic standards in several animal models. These studies provided evidence which indicated that bay-region diol epoxides are the most potent carcinogenic metabolites of these hydrocarbons. We formulated the "bay-region" theory which predicts that diol epoxides that have the epoxide group in the bay-region of the hydrocarbon will be the most chemically reactive and presumably biologically active for those hydrocarbons that are tumorigenic. To date studies from our laboratory as well as several other laboratories around the world have either proved or implicated bay-region diol epoxides as ultimate carcinogens for benzo[a]pyrene, benz[a]-anthracene, 7-methylbenz[a]anthracene, 7,12-dimethylbenz[a]anthracene, 3-methylcholanthrene, dibenz[a,h]anthracene, two dibenzpyrenes, chrysene, 5-methylchrysene, benzo[c]phenanthrene, and certain methylated cyclopentaphenanthrenes. The theory has stimulated considerable research in the field, all of which has been supportive of our initial concepts. Significant exceptions to the theory have yet to emerge.

One aspect of hydrocarbon-induced carcinogenesis which the bay-region theory made no attempt to take into account is that of relative and absolute stereochemistry of ultimate carcinogens. Much of our current efforts continue to address this question. Studies of the dihydrodiols and resultant bay-region diol epoxides formed by liver microsomes from benz[a]anthracene as well as phenanthrene and chrysene have shown that these molecules are all superimposable with the corresponding benzo[a]pyrene metabolites when their bay-regions are aligned. In the benzo[a]pyrene case, only one of four bay-region 7,8-diol-9,10-oxides exhibited strong tumorigenic activity, the predominant metabolically formed isomer. Tumor studies are continuing to determine if the related stereoisomer is also the active form from chrysene and benz[a]anthracene. Should this prove to be the case, the results would be suggestive that there is a highly chiral "receptor" for hydrocarbon carcinogens within the cell. Finally, studies have been initiated which will define the steric constraints of the active site of cytochrome P-450c, the principal oxidative enzyme responsible for the conversion of polycyclic aromatic hydrocarbons to ultimate carcinogens.

Synthetic Studies. Both *trans*-3,4-dihydroxy-3,4-dihydrobenz[a]anthracene and *trans*-1,2-dihydroxy-1,2-dihydrochrysene are known proximate carcinogens of their respective hydrocarbons. Synthesis of their (+)- and (-)-enantiomers as well as the diastereomeric pair of bay-region diol epoxides formed from each enantiomer when the double bond of the dihydrodiol ring is epoxidized either cis (isomer-1 series) or trans (isomer-2 series) to the benzylic hydroxyl group has been completed. For both hydrocarbons, (i) the tetrahydro analogues of the dihydrodiols were resolved by chromatographic separation of their diastereomeric bis-esters with (-)-menthoxyacetic acid, and the resultant tetrahydrodiols were converted to the requisite dihydrodiols, and (ii) the (+)-tetrahydrodiols led to the (-)-dihydrodiols, both with (R,R) absolute configuration. Assignment of absolute configuration in the chrysene series was achieved through application of the exciton chirality circular dichroism technique to the bis[p-(dimethylamino)benzoate of (-)-*trans*-1,2-dihydroxy-1,2,3,4-tetrahydrochrysene. NMR coupling patterns of the OCOCH$_2$O hydrogens in the bis-menthoxyacetic acid esters of these vicinal trans diols were found to be diagnostic of their absolute configuration. An interesting correlation was observed upon conversion of the enantiomerically pure dihydrodiols to their diastereomeric pairs of bay-region diol epoxides. As was previously the case for (-)-*trans*-(7R,8R)-7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene, the (-)-(R,R)-dihydrodiols in the chrysene and benz[a]anthracene series led to the (-)-diol epoxide-1 and (+)-diol epoxide-2 isomers. Of the four metabolically possible bay-region diol epoxides for each of the three hydrocarbons, tumor studies now indicate that the (+)-(R,S)-diol-(S,R)-epoxide-2 isomers (designated from the carbon bearing the benzylic hydroxyl group toward the epoxide) have practically all of the tumorigenic activity.

(+)-Benz[a]anthracene 10,11-oxide has been obtained in optically pure form by a four-step synthesis from (-)-*trans*-(10R,11R)-10-bromo-11-(menthyloxyacetoxy)-8,9,10,11-tetrahydrobenz[a]anthracene. Diastereoisomers were separated by recrystallization. The absolute configuration of (+)-arene oxide was established as (10S,11R) by application of the exciton-chirality method to an appropriate chemically related derivative. These optically active arene oxides will be used in evaluating the steric nature of the catalytic site of cytochrome P-450c.

Solvolytic Reactivity of Diol Epoxides and Related Compounds. The two conformationally rigid diastereomeric 9,10-oxides derived from *trans*-1,2,3,4,4a,10a-hexahydrophenanthrene have been synthesized, and their relative stereochemistries have been verified by X-ray structure determination of the acetate of the bromohydrin precursor of one of these epoxides. Inspection of molecular models indicates that one of these epoxides has the benzylic C-O bond of the epoxide ring aligned nearly parallel to the π orbitals of the aromatic ring and has the epoxide oxygen cis to the adjacent ring juncture hydrogen, whereas the other epoxide has this C-O bond not aligned with the π orbitals and has the epoxide oxygen trans to the adjacent ring juncture hydrogen. Rates of solvolysis of these two epoxides in 1:9 dioxane-water, ionic strength 0.1 (NaClO$_4$), at 25°C, follow the rate law $k_{obsd} = k_{H+}a_{H+} + k_o$, with similar values of k_{H+} for both diastereomers and a value of k_o for the aligned isomer that is ∿40 times larger than that for the nonaligned isomer. Upon solvolysis, the nonaligned isomer yields exclusively trans diol, whereas the aligned isomer yields predominantly cis diol (75%) under acidic conditions and ketone rearrangement product (∿85%) under neutral conditions. The predominant diol from acid hydrolysis of both isomers was postulated to arise from attack of solvent on the carbonium ion derived from benzylic C-O cleavage of the epoxide, to yield a pseudo-axial substituent. The observed chemistry of these rigid epoxides was used to explain conformational effects on the rates and products of solvolysis of the conformationally flexible benzo-ring diol epoxides derived from metabolism of polycyclic aromatic hydrocarbons: (1) For the hydronium ion catalyzed process (k_{H+}), rates are relatively insensitive to conformation, whereas product distribution is influenced (a) by the conformation of the carbonium ion that is initially formed, if this ion is relatively unstable, or (b) by the conformation of the carbonium ion that is preferred at equilibrium, if the ion is stable enough to undergo conformational equilibration prior to capture by solvent. (2) For the spontaneous process (k_o), the aligned conformation reacts considerably faster than the nonaligned conformation and also gives a carbonium ion with the required geometry for rearrangement to ketone. The observation that diol epoxides with the benzylic hydroxyl group cis to the epoxide ring (isomer-1 series) ordinarily react via the k_o process somewhat faster than their diastereomers with this hydroxyl group trans to the epoxide ring (isomer-2 series) and also yield ketone products suggests that the isomer-1 diastereomers probably react predominantly via the conformer of the carbonium ion that is related to the aligned conformation of the reactant; reaction via this slightly less favored ground-state conformation may provide the lowest energy pathway for generation of the ion. For the isomer-2 series the absence of ketone formation at neutral pH is consistent with reaction only via the conformer of the carbonium ion that is related to the nonaligned ground-state conformation.

As previously described, the two diastereomeric bay-region 3,4-diol-1,2-epoxides of benzo[c]phenanthrene represent the first example in which the hydroxyl groups of both diastereomers prefer the pseudo-diequatorial conformation. They also represent the first example for which both diastereomers are highly tumorigenic, about 15-fold more active than any other bay-region diol epoxide yet tested. These biological results are of particular interest in view of quantum mechanical calculations that predict these diol epoxides should also be among the least chemically reactive of those yet examined. Studies of the hydrolysis rate of these diol epoxides in 10% dioxane in water at 25°C with 0.1 M $NaClO_4$ present established that their disappearance follows the rate law $k_{obs} = k_{H+}a_{H+} + k_o$ as found for other diol epoxides and simple epoxides. In accord with the calculations, very long half-lives on the order of 500-1000 min were observed at pH 7. Similarly, both diol epoxides were found to be relatively unreactive toward sulfur and nitrogen nucleophiles. Our results for both S_N2 and solvolysis reactions indicate that these benzo[c]phenanthrene diol epoxides are the most chemically unreactive bay-region diol epoxides studied to date. Hence, their mutagenic and tumorigenic activity is not dependent upon high chemical reactivity in simple "model" reactions and must result from more specific aspects of their reactivity, possibly involving binding phenomena with cellular macromolecules.

Ortho-Hydroxylation and the NIH Shift. The NIH shift consists of the migration and retention of aromatic substituents when substituted aromatic hydrocarbons are ring-hydroxylated by monooxygenase enzymes. These novel reactions are mediated *via* arene oxide intermediates. Ortho-hydroxytoluene is a known fungal and hepatic metabolite of toluene which could arise either by a 1,2- or a 2,3-arene oxide of toluene. Since 2-deuterotoluene is known to be metabolized to 2-hydroxy-3-deuterotoluene by an NIH shift pathway, it had been proposed that the 2,3- and not the 1,2-arene oxide was the intermediate in this hydroxylation based on a postulated complete loss of deuterium from the initial carbocation derived from the 1,2-arene oxide. Both 2-deuterotoluene 1,2-oxide and 2-deuterotoluene 2,3-oxide have been synthesized and have been found to isomerize to ortho-hydroxytoluene with identical migration and retention of deuterium. Thus, although discrete and different carbocations may be drawn as initial intermediates in the isomerization of these two arene oxides to phenols, this constitutes a misrepresentation of the actual carbocation involved. Both arene oxides must open to a common delocalized carbocation from which the NIH shift occurs. Therefore, the NIH shift cannot be used as a criterion to distinguish between 1,2- and 2,3-arene oxides as intermediates in the hydroxylation of substituted benzenes. These results are of particular significance to the study of toxic pathways in the metabolism of substituted benzenes.

Stereoselective Metabolism of Enantiomeric Dihydrodiols to Bay-Region Diol Epoxides. Previously we had shown that the (+)-(7S,8S)-dihydrodiol of benzo[a]pyrene is stereoselectively metabolized to the 7,8-diol-9,10-epoxide isomer-1 in which the benzylic hydroxyl group is cis to the epoxide oxygen and that the (-)-(7R,8R)-dihydrodiol is stereoselectively metabolized to the 7,8-diol-9,10-epoxide isomer-2 in which these groups are trans. The latter pathway predominates in the animal to form the (+)-diol epoxide-2 isomer which is the only one of the four diol epoxide isomers with high tumorigenic activity. We have now extended these studies to the formation of bay-region diol epoxides of phenanthrene and benz[a]anthracene and have found that the same stereoselectivity pertains.

Enantiomerically pure isomers of *trans*-1,2-dihydroxy-1,2-dihydrophenanthrene have been obtained by chromatographic separation of their diastereomeric bis-esters with (-)-α-menthoxy-α-trifluoromethylphenylacetic acid. Liver microsomes from control rats, as well as from rats treated with phenobarbital or 3-methylcholanthrene, metabolize these dihydrodiols to a pair of diastereomerically related bay-region 1,2-diol-3,4-epoxides. In general, diol epoxide-1 was the major metabolite of the (+)-(1S,2S)-dihydrodiol, whereas diol epoxide-2 was the major metabolite of the (-)-(1R,2R)-dihydrodiol. The extent of this stereoselectivity is dependent on the source of the microsomes and is greatest for liver microsomes from 3-methylcholanthrene-treated rats; the ratio of diol epoxide-1 relative to diol epoxide-2 was 5.6:1 with the (+)-enantiomer as substrate and 1:5.5 with the (-)-enantiomer as substrate. For a given microsomal preparation, rates of metabolism were independent of the enantiomer composition of the substrate. Relative to microsomes from control animals, treatment of rats with 3-methylcholanthrene enhanced rates of metabolism by about 40%, whereas treatment with phenobarbital decreased rates to a similar extent when the amounts of metabolites formed per nanomole of cytochrome P-450 were compared. The failure of treatment by 3-methylcholanthrene to enhance markedly the rate of metabolism of a polycyclic aromatic hydrocarbon substrate is unusual.

Metabolism of the (+)-(3S,4S)- and (-)-(3R,4R)-enantiomers of *trans*-3,4-dihydroxy-3,4-dihydrobenz[a]anthracene has been examined with liver microsomes from control rats, as well as rats treated with 3-methylcholanthrene or phenobarbital and by a highly purified and reconstituted system containing cytochrome P-450c and epoxide hydrolase. In contrast to the metabolism of the related (-)-benzo[a]pyrene 7,8-, (-)-phenanthrene 1,2-, and (-)-chrysene 1,2-dihydrodiols from which >65% of the total metabolites constitute bay-region diol epoxides, very little (<16% of total metabolites) formation of bay-region diol epoxides was detected from (-)-benz[a]anthracene 3,4-dihydrodiol. For benz[a]anthracene, the (-)-(3R,4R)-enantiomer formed predominantly the (+)-3,4-diol-1,2-epoxide-2 diastereomer which displays high tumorigenic activity. Studies on the (+)-(3S,4S)-enantiomer failed to provide evidence for the formation of bay-region diol epoxides and thus provide the first example where liver microsomes form very little, if any, diol epoxide from a dihydrodiol whose hydroxyl groups are predominantly pseudo-diequatorial. Major metabolites of both enantiomers consisted of bis-dihydrodiols formed via the action of epoxide hydrolase on dihydrodiol-arene oxide precursors. A novel microsomal metabolite, benz[a]anthracene 3,4-quinone, was also identified. Antibody experiments established that the quinone is formed predominantly by the NADPH-supported cytochrome P-450-dependent monooxygenase system in liver microsomes from 3-methylcholanthrene-treated rats, presumably via a mechanism involving molecular oxygen. In addition, evidence for an NAD-supported pathway which does not involve cytochrome P-450c was also detected.

Biological Activity of Dibenzopyrene Derivatives. We have previously described the synthesis of dihydrodiols and bay-region diol epoxides of dibenzo[a,h]pyrene (DB[a,h]P) and dibenzo[a,i]pyrene (DB[a,i]P), two carcinogenic environmental contaminants related to benzo[a]pyrene. Testing of the mutagenic and tumorigenic potential of these compounds is now complete with the result that bay-region diol epoxide metabolites are probably responsible for most of their biological activity.

The mutagenic activities of DB[a,h]P, DB[a,i]P, and a total of 11 of their benzo-ring derivatives were evaluated in bacterial and mammalian cells in the absence or presence of a mammalian metabolic activation system. *trans*-1,2-Dihydroxy-1,2-dihydrodibenzo[a,h]pyrene and *trans*-3,4-dihydroxy-3,4-dihydrobenzo-[a,i]pyrene, the expected dihydrodiol precursors of bay-region diol-epoxides, were metabolized by a cytochrome P-450-dependent monooxygenase system to products which were more mutagenic to strains TA98 and TA100 of *Salmonella typhimurium* than were the metabolic products formed from their respective parent hydrocarbons. For each dihydrodiol, replacement of the benzo-ring double bond adjacent to the diol moiety with a single bond resulted in tetrahydrodiol derivatives which could not be metabolically activated, suggesting that one or both diastereomeric bay-region diol epoxides were the bioactivated metabolites. The authentic bay-region diol epoxide diastereomers of DB[a,h]P and DB[a,i]P in which the benzylic hydroxyl group and the epoxide oxygen are *trans* (diol epoxide 2 series) were highly mutagenic in strains TA98 and TA100 of *S. typhimurium* and in cultured Chinese hamster V79 cells. Neither diol epoxide was significantly, if at all, metabolized by epoxide hydrolase. The bay-region diol epoxide of DB[a,i]P was from 1.5 to 5 times more active as a mutagen than the diol epoxide of DB[a,h]P, and in strain TA98 of *S. typhimurium* as well as Chinese hamster V79 cells, it had activity comparable to that of the highly carcinogenic bay-region diol epoxide of benzo[a]pyrene. Notable, the high biological activity of these diol epoxides is paralleled by their high chemical reactivity.

DB[a,h]P, DB[a,i]P, and seven of their benzo-ring derivatives were tested for tumorigenic activity on mouse skin and in newborn mice. In the tumor studies on mouse skin, a single topical application of 50, 200, or 600 nmol of compound was followed 7 days later by twice-weekly applications of the tumor promotor 12-O-tetradecanoylphorbol-13-acetate for 16 or 24 weeks. With the exception of 2,10-difluorodibenzo[a,i]pyrene, all of the compounds had significant tumor-initiating activity at all doses tested. *trans*-1,2-Dihydroxy-1,2-dihydrobenzo-[a,h]pyrene and *trans*-3,4-dihydroxy-3,4-dihydrodibenzo[a,i]pyrene, the metabolic precursors of bay-region diol epoxides, had tumor-initiating activity that was equivalent to their parent hydrocarbons. Saturation of the double bond in the benzo-ring of these dihydrodiols resulted in the formation of tetrahydrodiols whose tumor-initiating activity was not significantly different from that observed with the corresponding dihydrodiols at the 50-nmol dose. The bay-region diol epoxides of DB[a,h]P and DB[a,i]P, in which the benzylic hydroxyl group and the oxirane oxygen are *trans* (isomer-2), induced significantly fewer tumors per mouse than did their dihydrodiol and parent hydrocarbon precursors.

In the tumorigenicity study in newborn mice, a total dose of 87.5 nmol of the hydrocarbon divided into three i.p. injections was administered on the first, eighth, and 15th day of life, and tumorigenic activity was determined when the mice were 49 to 54 weeks old. *trans*-1,2-Dihydroxy-1,2-dihydrodibenzo[a,h]pyrene and *trans*-3,4-dihydroxy-3,4-dihydrodibenzo[a,i]pyrene induced 3- to 8-fold more pulmonary tumors per mouse and 4- to 5-fold more hepatic tumors per male mouse than the respective parent hydrocarbons. The corresponding tetrahydrodiols had no more than one-eighth of the pulmonary tumorigenic activity of the corresponding dihydrodiol. The bay-region diol epoxide (isomer-2) of DB[a,h]P had tumorigenic activity equal to the parent hydrocarbon but significantly less than its dihydrodiol precursor. The bay-region diol-epoxide (isomer-2) of DB[a,i]P was highly toxic, and only 19% of the mice survived to termination of the study. This diol epoxide had significantly less tumorigenic activity towards the lung than did either its dihydrodiol precursor or the parent hydrocarbon. Notably, 20% of the surviving mice treated with the diol epoxide of DB[a,i]P had leukemia at the termination of the study. 2,10-Difluorodibenzo[a,i]pyrene had no tumorigenic activity in newborn mice at the single dose tested.

Stereoselective Formation of Arene Oxides by Cytochrome P-450c. The facial selectivity of purified rat liver cytochrome P-450c toward epoxidation of benzo[a]pyrene (BP) at its 4,5-position (K-region) was determined by formation, separation and quantitation of the diastereomeric trans-addition products of glutathione with enzymatically produced benzo[a]pyrene 4,5-oxide (BP 4,5-oxide). Non-enzymatic trans addition of glutathione to synthetic (+)-(4S,5R)- and (-)-(4R,5S)-BP 4,5-oxides occurs equally at carbons 4 and 5 to give two diastereomeric pairs of positional isomers. The position of the glutathionyl moiety in each isomer was determined by acetylation, oxidation of the thioether to the sulfoxide, *cis*-elimination of the sulfoxide, and identification of the acetoxybenzo[a]pyrene formed. Correlation of the glutathione conjugates obtained from BP 4,5-oxide derived from cytochrome P-450c catalyzed oxidation of BP with those obtained from the synthetic enantiomers indicated that $\geq$97% of the enzymatically formed arene oxide was the (+)-(4S,5R) enantiomer.

The principal oxidative metabolites formed from benz[a]anthracene (BA) by the rat liver microsomal monooxygenase system are the 5,6- and 8,9-arene oxides. In order to determine the enantiomeric composition and absolute configuration of these metabolically formed arene oxides, an HPLC procedure has been developed to separate the six isomeric glutathione conjugates obtained synthethically from the individual enantiomeric arene oxides. Both (+)- and (-)-BA 5,6-oxides gave the two possible positional isomers, but only one positional isomer was formed in each case from (+)- and (-)-BA 8,9-oxide. When [^{14}C]-BA was incubated with a highly purified and reconstituted monooxygenase system containing cytochrome P-450c, and glutathione was allowed to react with the arene oxides formed, radioactive adducts were formed predominantly (>97%) from the (+)-(5S,6R) and (+)-(8R,9S) enantiomers. These and the above results are being used to formulate a theoretical model of the catalytic binding site of cytochrome P-450c.

Reactions of Bay-Region Diol Epoxides with Nucleic Acids. Chemical modification of DNA is generally held to be the mechanism by which carcinogens express their tumorigenic activity. Because of our findings of high stereoselectivity in this process as initiated by the polycyclic hydrocarbons, we have reinstituted studies in this area. Both chemical mechanism studies and computer assisted modeling of reactions are in progress. Initial experiments have shown that 5'-guanosine monophosphate in the monohydrogen form ($GMPH^-$) exhibits pronounced general acid catalysis in the hydrolysis of the bay-region diol epoxides of benzo[a]pyrene to inactive tetraols under conditions where guanosine is unreactive. Although the pK_a values for $GMPH^-$ and dihydrogen phosphate ion are very similar, $GMPH^-$ is 60-80 times more effective, possibly *via* prior complex formation. This result suggests the possibility of selecting or designing other compounds that react rapidly with diol epoxides by a similar mechanism and may be capable of inactivating these carcinogens *in vivo*.

Precocene I. The anti-juvenile hormone precocene I (7-methoxy-2,2-dimethyl-2H-benzo[b]pyran) has the potential to become a widely used insecticide. Cis and trans 3,4-diols are known insect metabolites which probably arise via a potentially toxic 3,4-oxide. Thus, we have initiated studies of the stereoselectivity of the hepatic metabolism of this naturally occurring plant isolate. Both the cis and trans diols have been prepared in optically pure form by diastereomer resolution of their bis-esters with (-)-menthoxyacetic acid. Assignment of absolute configuration was achieved by independent nuclear magnetic resonance and circular dichroism techniques. The cis and trans diols have been identified as major metabolites with liver microsomes from 3-methylcholanthrene treated rats. Details of the stereoselectivity of the process are under investigation.

SECTION ON PHARMACODYNAMICS

Pharmacologically Active Compounds from Amphibians and Other Natural Sources

Skin extracts from the Colombian poison-dart frog *Phyllobates terribilis* contain three major steroidal alkaloids: Batrachotoxin, homobatrachotoxin and batrachotoxinin A. Minor congeners of batrachotoxin and homobatrachotoxin containing a 4β-hdyroxy substituent are identified based on mass spectra and proton and carbon-13 magnetic resonance spectra. Two "indole alkaloids" *ℓ*-calycanthine and *d*-chimonanthine, enantiomeric to the same compounds from plants, and noranabasamine (5-(2-piperidyl)-2,3'-dipyridine), a des-N-methyl analog of the plant alkaloid anabasamine, are present as minor constituents. Futher quantities of batrachotoxins, pumiliotoxins, histrionicotoxins and gephyrotoxins have been isolated from skin extracts of neotropical frogs for the investigation of structure and pharmacological properties. The N-methyl and quaternary N,N-dimethyl derivatives of histrionicotoxin have been prepared.

Structures of three further members of the histrionicotoxin class (2,7-disubstituted 1-azaspiro[5.5]undecan-8-ols) of dendrobatid alkaloids were defined based on mass and nuclear magnetic resonance spectral properties. Carbon-13 magnetic resonance spectral assignments for histrionicotoxin, six natural congeners, perhydrohistrionicotoxin and 8-deoxyperhydrohistrionicotoxin were established.

The structure 3-butyl-5-propylindolizidine, tentatively assigned to a minor alkaloid in skin extracts from a number of poison frogs of the neotropical genus *Dendrobates*, has been confirmed and its stereochemistry determined as 5E, 9E by comparison on gas chromatography-mass spectrometry with the four synthetic diastereomers.

The absolute stereochemistry of pumiliotoxin B has been elucidated. The E-configuration of the double bond and the *threo* relationship for the diol moiety of the side chain were determined, respectively through nuclear-Overhauser effects and model *threo*- and *erythro*-diols.

The sodium channel specific agent batrachotoxin (BTX) has been shown to induce a time- and concentration-dependent depolarization of a vesicular preparation from guinea pig cerebral cortex (microsacs). The $K_{0.5}$ for depolarization by BTX was 0.011 μM at 30 min. Membrane potential was determined by the equilibrium distribution of triphenyl-[^{3}H]methyl phosphonium ion. A series of seven local anesthetics were shown to competitively inhibit BTX induced depolarization with K_i values ranging from 0.9 μM for dibucaine to 780 μM for lidocaine ethiodide. The specific binding of labeled batrachotoxinin-A 20-α-benzoate ([^{3}H]BTX-B) to voltage-sensitive channels in microsac preparations from mouse cerebral cortex in the presence of scorpion toxin was measured and found to yield a range of K_d values of about 25 to 30 nM and B_{max} values of about 1.0 picomoles/mg; in the preparation from guinea pig cerebral cortex it was found to yield K_d values of about 55 nM and B_{max} values of about 2.2 picomoles/mg. A series of 14 local anesthetics were shown to inhibit the specific binding of [^{3}H]BTX-B with K_i values ranging from 0.8 μM for dibucaine to 500 μM for benzocaine. The rank order of potency of the local anesthetics as antagonists of [^{3}H]BTX-B binding was as follows: dibucaine > tetracaine > bupivacaine > diphenhydramine > piperocaine > cocaine > procaine > lidocaine > benzocaine. The quaternary local anesthetic QX-572 was comparable in its potency to tetracaine. The rank order and relative potency of the local anesthetics tested in both paradigms were similar with the exception of lidocaine ethiodide, which was 16 times more potent as an inhibitor of [^{3}H]BTX-B binding than it was as an inhibitor of BTX-elicited depolarization.

The binding of labeled batrachotoxinin-A benzoate (BTX-B) to voltage-sensitive sodium channels in broken membrane preparations of mouse cerebral cortex has been measured as a function of the pH. Specific binding is negligible at pH <6.0, maximum at pH 8.5, and decreases again at pH 9.0. A major component of nonspecific binding, however, increases linearly in the pH range 8.0-9.0. The pK_a of batrachotoxinin-A, an analogue of BTX-B, was found by titrimetric methods to be $\geq$8.2. Analysis of the data shows that at least part of the pH dependence of BTX-B binding is due to the titration of a sodium channel residue(s) associated in some way with the BTX-B recognition site. The possible involvement of a histidine residue is suggested.

Pumiliotoxin B (PTX-B) causes a significant increase in the amplitude and duration of the single muscle contraction in frog, rat, and normal chicken muscles. In the presence of PTX-B, the tetanic tension induced during high-frequency stimulation was potentiated and fusion tension occurred at lower-than-normal rates of stimulation. In addition, the decay phase or relaxation of the muscle after tetanic stimulation was markedly prolonged and the decay showed several different components or decay rates. The toxin produced increases in evoked release of transmitter and multiple EPP discharge after a single nerve shock. Furthermore, the toxin blocked Ca-ATPase. In the normal chicken PLD muscles, the toxin had a qualitatively similar effect on the muscle twitch and tetanus as compared with the frog. However, when the dystrophic chicken muscle was exposed to 1.5 μM PTX-B, no effect on single muscle twitch was recorded and only slight prolongation of the decay phase after attainment of tetanus was observed. The presynaptic effect of the toxin was similar to that seen in the frog, but PTX-B had no effect on the nerve ending of dystrophic chicken skeletal muscle. Finally, PTX-B blocked the Ca-ATPase of normal chicken muscle but not that of dystrophic chicken muscle seen at concentrations of 30 μM. Because PTX-B did not affect Na, K, or Cl^- conductances, the action of the toxin on the presynaptic nerve terminal may be explained by removal and competitive displacement of Ca from its active sites.

Pumiliotoxin-B (PTX-B), an indolizidine alkaloid isolated from the neotropical frog *Dendrobates pumilio*, is a potent and specific inhibitor of the Ca^{++}-Mg^{++}-ATPase of skeletal muscle sarcoplasmic reticulum (SR). The PTX inhibition of SR ATPase activity is dependent on the ratio of toxin to protein, with 50% inhibition occurring at ca. 650 PTX-B/ATPase. The inhibition is enhanced significantly by an increase in temperature from 25 to 37°C. PTX-B decreases the absolute value of the slope of the Arrhenius plot for ATPase activity. PTX-B inhibition is antagonized by an increase in either ATP or Ca^{++} concentration. Increasing concentrations of ATP cause parallel changes in catalytic activity and loss of sensitivity to the toxin. Thus, interaction of ATP with a regulatory site alters the sensitivity to PTX-B. On the other hand, higher concentrations of Ca^{++} are required to overcome inhibition than to stimulate ATPase activity. It therefore appears that Ca^{++} interacts with sites other than the high affinity binding site(s) on the ATPase to decrease sensitivity to PTX-B. Inhibition with respect to ATP is mixed linear noncompetitive with K_i = 14 μM at 37°C and 1.25 μM free Ca^{++}. The Hill coefficient for ATP is unaltered by PTX-B, suggesting that PTX-B does not alter interaction between catalytic and proposed regulatory sites for ATP. The data indicate that PTX-B does not directly compete for either the Ca^{++} or ATP site on the ATPase, however, PTX-B markedly decreases the affinity of the enzyme for ATP. The lipophilic nature of PTX-B, the dependence of inhibition on protein concentration, and the reduction of inhibition by high Ca^{++} concentrations suggest that the action of the toxin may be related to alterations in the membrane environment of the ATPase.

Pumiliotoxin-C (PTX-C), a *cis*-decahydroquinoline alkaloid and synthetic analogs, inhibited direct and indirect elicited twitches in the rat phrenic nerve diaphragm preparation. At 40 μM, the most potent analog, 2,5-di-n-propyl-*cis*-decahydroquinoline (PTX-C_{II}), reversibly blocked the indirect elicited twitch and markedly depressed the amplitude of endplate potentials and minature endplate potentials without affecting muscle membrane potential. PTX-C_{II} did not depress the direct elicited twitch in diaphragm muscle treated with α-bungarotoxin. At 40 μM, the compound blocked extrajunctional acetylcholine (ACh) sensitivity in chronically denervated soleus muscle of the rat. The peak amplitude of the endplate current in frog sartorius muscle was depressed by PTX-C_{II} at both positive and negative membrane potentials. Nonlinearity in the current-voltage relationship appeared at membrane potentials greater than -100 mV with concentrations of PTX-C_{II} from 2.5 to 7.5 μM. The decay phase of the endplate current was shifted to lower values such that it decayed faster at all membrane potentials but retained voltage sensitivity. PTX-C_{II}, the most potent of the four, inhibited binding of $[^3H]$perhydrohistrionicotoxin to *Torpedo* electroplax membranes with an IC_{50} value of 1.5 μM in the absence of carbamylcholine (CARB) and 0.1 μM in the presence of CARB. The other analogs were less potent, but all showed higher affinities in the presence of CARB. PTX-C_I, -C_{II} and -C_{III} had no effect on the binding of $[^{125}I]$α-bungarotoxin to ACh receptors in *Torpedo* membranes while slightly enhancing the binding of $[^3H]$ACh whereas PTX-C_{II} gave slight inhibition of both receptor ligands at concentrations >10 μM. The pumiliotoxin-C class of akaloids therefore appear to block neuromuscular transmission primarily *via* interactions with sites associated with the ion channel controlled by the ACh receptor.

Gephyrotoxin inhibits neuromuscular transmission by interaction with sites on the ionic channel associated with acetylcholine receptors. It shares this propertyl with histrionicotoxin, pumiliotoxin C, phencyclidine, and certain local anesthetics. However, the site at which gephyrotoxin acts does not appear identical with the histrionicotoxin site based on binding studies. Gephyrotoxin is many fold less potent in displacing [^{3}H]histrionicotoxin than in displacing [^{3}H]phencyclidine. The results suggest the presence of differing domains of the site by which such compounds block acetylcholine-regulated channels in muscle endplates. A wide series of gephyrotoxins (bicylic and tricyclic) histrionocotoxins and pumiliotoxins have been screened versus binding of [^{3}H]histrionicotoxin and [^{3}H] phencyclidine to closed and open forms of the acetylcholine-receptor channel complex in *Torpedo* electroplax membranes. Terminal double bonds appear to confer high potency to the histrionicotoxins.

Pharmacodynamic Amines and Enzymes Involved in Their Metabolism

Catechol-O-methyltransferase. Specific antibodies to rat liver catechol-O-methyltransferase (COMT) are continuing to be utilized to determine the localization of COMT at the cellular and subcellular level; to measure the rate of synthesis and degradation of this enzyme; to measure and identify COMT in various hybrid cell lines; to measure and localize this enzyme in animal and human breast tumors (adenocarcinomas); to follow the changes in COMT localization in the developing placenta in the course of both normal and delayed implantation in rat uterus; and to characterize the increase in enzyme level in ductal cells of the lactating rodent.

The distribution of COMT in the CNS of rat and other rodents has been studied in detail and a generalized theory of the function of COMT has been proposed. At present the role of COMT has been divided into two general categories: 1) dealing especially with the restriction of catechol diffusion and transport between fluid compartments both in the CNS and the periphery, and 2) the control of catechol steroid levels in the developing placenta and the ductal cells of the lactating mammal.

The distribution, level and characterization of the cellular locus of hormonally sensitive COMT is being investigated in the pregnant rat uterus, under conditions where implantation of the fertilized blastocyst is delayed by administration of exogeneous hormones, and by mating lactating rats on the first day post-partum. A possible role of COMT accompanying the degenerative changes in the endometrial epithelial cells preceding implantation has been proposed. This study has been extended to include an examination of rat oviduct. Here, as in uterus proper, the primary site of COMT is in the cytoplasm of the epithelial layer lining the oviduct. COMT-containing cells with the rat ovary have been tentatively identified. The examination of the relationship between epithelial COMT and hormonal events in pregnancy is being expanded to include measurement of estrogen and hydroxylases. Preliminary studies are underway using an antisera to catecholsteroids to measure to concentration of these catechols and perhaps their localization at selected periods during pregnancy.

Studies on the distribution and level of COMT under normal hormonal stimuli (lactation) in rat, mouse and human breast are in progress. The level of COMT measured by biochemical and by immunochemical techniques appears to increase in the ductal cells during lactation and decline with the cessation of lactation. No COMT was observed in secreting cells of the rodent or human breast.

The localization of COMT in various tumors of both rodent and human breast have been investigated. It is evident that COMT levels are elevated in both rodent (transplantable) and human adenocarcinomas of the ductal tissues of breast. The technology of the cellular localization of COMT by immunofluorescence and immunoperoxidase have been improved both as regards specificity and sensitivity. Because of the possible clinical importance of this observation in human breast cancer a large number of tumors of known history and diagnosis are being surveyed with regard to the presence of elevated COMT levels.

Attempts to map the chormosomal location of human and rodent COMT have been initiated. Rabbit antisera against rat COMT and against human COMT have been used to localize the position of immunoreactive proteins in polyacrylamide gels following transfer to nitrocellulose paper. Purified rat liver COMT showed a single band on SDS-PAGE (MW 26000) and yielded two immunoreactive bands after isoelectric focusing, pI's 5.2 and 5.3. Similar patterns were obtained with: lysates of human red blood cells; partially purified COMT from human kidney and human neuroblastoma cells. Preliminary results obtained with antibody to human hepatic COMT are similar to those obtained with antisera to rat COMT. At present our results suggest that COMT exists as a single molecular weight species in at least two forms differing in net charge. These forms are present in both human and rat cells. Chromosomal mapping studies with somatic cell hybirds have been initiated.

The turnover of COMT in rat has been investigated. The apparent turnover is very slow. In order to economize on the use of isotope and antisera, turnover studies are being carried out in mice.

Fluorine-Substituted Biogenic Amines. The progress to date on the synthesis and biological properties of ring fluorinated biogenic amines emphasized the special advantages of the substitution of fluorine for hydrogen in the design of analogs of biologically important compounds. The small size and high electronegativity of fluorine allow many such analogs to be useful pharmacological and medicinal agents. Many biogenic amines have ionizable groups which could reflect directly the electronegative effects of fluorine substitution. This consideration prompted us to investigate synthetic methods for the ring-fluorination of a series of biogenic amines. Extensive biological evaluation of these fluorinated amines has revealed several fluoroamines with significantly altered biological properties. To date, the most interesting results have involved the adrenergic agonists specificities of norepinephrine (NE). While 5-FNE behaves like NE, 6-FNE is essentially a pure α-adrenergic agonist and 2-FNE is a pure β-adrenergic agonist. Altered adrenergic specificities were observed with the fluoro derivatives of the classical β-and α-agonists isoproterenol and phenylephrine. At present several studies are in progress to determine the rational for the changes in affinity of norepinephrine (and related compounds) for specific binding sites on macromolecules due to the presence of the fluorine substituent.

The extent of *meta*- and *para*-O-methylation by COMT of 2-fluoro-, 5-fluoro- and 6-fluoronorepinephrine (F-NE) at pH 7 and 9 was determined. The rank order of preference for *para*-O-methylation is 5-F-NE >> NE > 6-F-NE > 2-F-NE. In all cases increasing the pH to 9 results in an increase in *para*-O-methylation. Results with 2- and 5-F-NE demonstrate the importance of ionization in the methyltransferase reaction when fluorine is situated *ortho* to one of the phenolic groups. To establish unequivocally the identities of the products, the isomeric, monofluorinated vanillins and isovanillins were synthesized and directly related to the products formed enzymatically from the monofluorinated norepinephrines. In order to facilitate this approach, a high pressure liquid chromatography system has been developed which has permitted a marked increase in the speed, accuracy and sensitivity of assay of both COMT-activity and the separation of the methylated products. The effects of pH on the distribution of the O-methylated products of norepinephrine suggest that the ionization of the sulfhydryl group at the active site of COMT may alter the binding mode of the substrate at higher pH values and subsequently bring the *para*-hydroxy group in a more favorable position for methylation.

2-, 5-, and 6-Fluoroisproterenols were synthesized by reduction of the Schiff base formed between the corresponding ring-fluorinated 3,4-dibenzyloxyphenethanolamine and acetone, followed by reductive debenzylation in the presence of oxalic acid to yield crystalline neutral oxalates. The apparent beta-adrenergic potencies were determined in the isolated guinea pig atria. 2-Fluoro and 5-fluoroisoproterenol were equipotent with (±)isoproterenol while 6-fluoroisoproterenol was virtually inactive. No alpha-adrenergic agonist activity (guinea pig aorta) was shown by any of the fluoroisoproterenols. Displacement of alpha- and beta-specific radioligands from isolated membrane preparations from rat brain by the fluoroisoproterenols were in agreement with the responses of the organ preparations. Thus the apparent fluorine induced specificity is due to specificity at the receptor binding site. The effects of fluorine substitution are discussed with regard to the apparent negative influence of the 6-fluoro substituent on the beta-agonist properties of isoprotereonl; the lack of any increase in potency due to the 2-fluoro substituent and the possibility of fluorine-induced changes in the electron density of the aromatic ring as a possible rational for the fluorine-induced specificity of both the fluoroisoproterenols and the fluoronorepinephrines.

The synthesis of the amino acid precursors of 6-fluorodopamine and 6-fluoro-α-methyldopamine, 6-fluoro-DOPA and 6-fluoro-α-methyl-DOPA has been completed. Preliminary studies indicate that both amino acids cross the blood brain barrier and undergo decarboxylation to the respective amines. Both lower blood pressure in the intact SHR rat. The 6-fluoroamines have a much lower affinity for COMT than the parent compounds or the 2- or 5-fluoro derivatives. Thus we expect the 6-fluoro-α-methyl DOPA and/or 6-fluoro-α-methyl dopamine to have a much greater biological half-life than the related parent compounds. If true, this is of importance with regard to the possible clinical applications of the compound and its use as a fluorine marker *in vivo*.

A study of the chemical environment of different cellular pools of fluorine-19 labeled compounds *in situ* has been initiated using nuclear magnetic resonance spectroscopy (NMR). The sensitivity of NMR to fluorine is second only to proton and tritium at constant field strength. This together with the virtual absence of endogenous fluorine makes it an optimum nucleus for NMR studies. This technique was used successfully to examine the uptake of 6-fluorodopamine by synaptosomes from rat striatum. By repetitive scanning, a clear fluorine signal was obtained from synaptosomes preincubated with 10^{-6}M 6-fluorodopamine. The effects of temperature, inhibitors of dopamine metabolism, dopamine uptake and the effects of reserpine on dopamine binding sites were examined. Fluorine signals corresponding to the expected metabolites were observed. The width of the 6-fluorodopamine signal was increased in the presence of metabolic inhibitors and dramatically decreased by reserpine. Broadening of the peak is a measure of restriction of motion and presumably a measure of 6-fluorodopamine binding within the storage vesicles whereas the narrowing of the peak indicates an increase in the concentration of unbound, free compound. The metabolites all yielded narrow peaks suggesting unrestricted motion. The identity of the metabolites were confirmed by HPLC with authentic compounds.

Calcium-Dependent Release of [^{3}H]Norepinephrine and [^{3}H]Dopamine. A simple gravity-flow perfusion technique employing Sephadex G-10 microcolumns was used to measure tritium release from a norepinephrine-labeled vesicular preparation from guinea pig cerebral cortex. Release of radioactive norepinephrine evoked by high concentrations of K^+ or by veratridine is absolutely dependent on the presence of external calcium ions. Submicromolar concentrations of norepinephrine or clonidine inhibit K^+- or veratridine-evoked release *via* interaction with alpha-adrenergic receptors. The inhibition is progressively overcome by increasing concentrations of calcium ions. Two agents which affect cyclic AMP-generating systems were examined for effects on K^+-evoked release of norepinephrine. The phosphodiesterase inhibitor ZK 62771 slightly elevates cyclic AMP levels in the brain vesicular preparations, while the diterpene forskolin which is a general activator of adenylate cyclases markedly elevates cyclic AMP levels. Neither agent alone has consistent effects on release of norepinephrine, but a combination of ZK 62771 and forskolin markedly reduces K^+-evoked release of norepinephrine. This inhibition is overcome by increasing concentrations of calcium ions. The results suggest that high levels of cyclic AMP may be inhibitory to K^+-evoked release of norepinephrine from brain vesicular entities.

Adenosine analogs such as 2-chloroadenosine, adenosine 5'-cyclopropylcarboxamide, N^6-D-phenylisopropyladenosine (D-PIA), N^6-L-phenylisopropyladenosine (L-PIA), and N^6-cyclohexyladenosine inhibit the calcium-dependent K^+-evoked release of [^{3}H]norepinephrine from guinea pig cerebral cortical and hippocampal vesicular preparations. The inhibitions require concentrations of adenosine analogs in the 10-100 μM range. D-PIA appears somewhat more active than L-PIA. Inhibition by 2-chloroadenosine is blocked by theophylline and is reduced in the presence of high concentrations (2 mM) of extracellular calcium ions. Apomorphine antagonizes calcium-dependent K^+-evoked release of [^{3}H]dopamine from guinea pig striatal vesicular preparations. Adenosine analogs cause both potentiation and inhibition of K^+-evoked release of [^{3}H]dopamine. At low concentrations (0.15 mM) of extracellular calcium D-PIA, L-PIA and adenosine 5'-cyclopropylcarboxamide potentiate release, while having no effect at high concentrations of calcium. 2-Chloroadenosine inhibits release at both low and high concentrations of calcium. 8-Bromoadenosine has no effect on release of either [^{3}H]norepinephrine or [^{3}H] dopamine. Structure activity relationships for inhibition of release of catecholamines by adenosine analogs from these brain preparations are not those expected for either A_1 (high affinity) or A_2 (low affinity) adenosine receptors.

The calcium antagonists, D-600 and diltiazem, inhibit calcium-dependent K^+-evoked release of [^{3}H]norepinephrine from guinea pig cerebral cortical vesicular preparations, and of [^{3}H]dopamine from striatal vesicular preparations. The inhibition is reduced in the presence of high concentrations (2 mM) of extracellular calcium. At 0.15 mM calcium, D-600 and diltiazem have biphasic effects on release of [^{3}H]dopamine, being inhibitory at lower concentrations (<10 μM) and stimulatory at higher concentrations. Nifedipine and dantrolene had no significant effects on K^+-evoked release of [^{3}H]norepinephrine.

The Role of Cyclic Nucleotides in the Nervous System

Forskolin, a novel diterpene activator of adenylate cyclase in membranes and intact cells, activates the enzyme in membranes from mutant cyc^- S49 murine lymphoma cells and the soluble enzyme from rat testes. Each of these enzymes consists only of the catalytic subunit and does not have a functional guanine nucleotide-binding protein. In both cases forskolin converts the manganese-dependent enzymes to a form which does not require manganese for activity. Forskolin can also stimulate a detergent-solubilized preparation of adenylate cyclase from rat cerebral cortex. Activation of adenylate cyclase by forskolin is therefore not dependent on a perturbation of membrane structure nor does it require a functional guanine nucleotide-binding subunit.

Adenylate cyclase in rat striatal membranes is inhibited by GppNHp when assayed in the presence of the adenylate cyclase activator forskolin. The forskolin-activated enzyme is inhibited about 30% at low concentrations of GppNHp (0.01-1 μM). These concentrations of GppNHp have no effect on the basal activity of the enzyme. Higher concentrations of GppNHp, which alone stimulate the basal activity, have no further inhibitory effect on the forskolin-activated enzyme. GppNHp also inhibits forskolin-activated adenylate cyclase in membranes from cultured rat thyroid cells and rat cerebral cortex. These adenylate cyclase systems are subject to hormonal stimulations as well as hormonal inhibitions and presumably contain both the stimulatory guanine nucleotide regulatory subunit (N_s) and the putative inhibitory guanine nucleotide regulatory subunit (N_i). GppNHp and forskolin have additive stimulatory effects on adenylate cyclase in membranes from wild type S49 lymphoma cells and no inhibition by GppNHp is observed. Adenylate cyclase in these cells does not exhibit hormonal inhibitions and is considered a simple stimulatory system, i.e. it does not contain N_i subunits. The inhibition by GppNHp of forskolin-activated adenylate cyclase in striatal membranes is persistent, is not competitive with forskolin, is not additive with GTP-inhibition and can be antagonized by GDP-βS. $MnCl_2$ at low concentrations (1 mM) blocks the GppNHp-mediated inhibition, allowing a GppNHp-augmentation of the forskolin-stimulated activity. It is concluded that GppNHp inhibits forskolin-activated adenylate cyclase via its binding at a guanine nucleotide binding site associated with the N_i subunit. Thus, GppNHp-inhbition of forskolin-activated adenylate cyclase is proposed to be a characteristic of adenylate cyclase systems which contain functional N_i subunits.

The diterpene forskolin, a general activator of adenylate cyclase, is a potent inhibitor of aggregation of hyman platelets. Complete inhibition of aggregation in platelet-rich plasma occurs at 10-20 μM forskolin. The IC_{50} values for forskolin *versus* ADP-induced and arachidonate-induced aggregation are 6 μM and 0.6 μM, respectively. Prostaglandins (PG) which activate platelet adenylate cyclase *via* specific receptors are also more potent *versus* arachidonate-induced aggregation. Forskolin markedly augments the ability of the prostaglandins PGI_2, PGD_2, and PGE_1 to inhibit platelet aggregation. Forskolin (ED_{50} 4-5 μM) elevates ^{3}H-labeled cyclic AMP levels in [^{3}H]adenine-labeled washed platelets by 30- to 40-fold. This response is near maximal after 2 min and is rapidly reversible. The presence of PGI_2, PGE_1, or PGD_2 increases both the efficacy and the potency of forskolin as an activator of cyclic AMP-generating systems. Conversely, a low concentration of forskolin (0.8 μM) markedly increases both the efficacy and the potency of PGI$_2$, PGE$_1$, and PGD$_2$ as activators of cyclic AMP-generating systems. This concentration of forskolin also augments the elevation of cyclic AMP elicited by PGE$_{1\alpha}$, 6-keto-PGE$_{1\alpha}$, PGE$_2$, and 2-chloroadenosine. Isoproterenol has no effect on cyclic AMP in the presence or absence of forskolin, whereas norepinephrine inhibits the forskolin-induced elevation of cyclic AMP. There is an excellent correlation between the effect of PGE$_1$ or forskolin on cyclic AMP levels and their inhibitory effect on platelet aggregation. The physiological effects of forskolin on human platelets appear to be mediated both by direct stimulation of adenylate cyclase and through a marked enhancement of receptor-mediated stimulation of the enzyme.

The effect of the diterpene forskolin on vascular permeability alone and in combination with bradykinin, prostaglandin E$_1$, adenosine or histamine has been investigated in rats. Vascular permeability in rat skin was measured using ^{125}I-labelled bovine serum albumin as a tracer. In addition, the effect of forskolin on footpad edema induced by the injection of a mixture of 2% carrageenin was determined. Forskolin caused a marked potentiation of the increase in vascular permeability in rat skin elicited by the intradermal injection of histamine or bradykinin. However, forskolin caused a significant suppression of the prostaglandin E$_1$-induced vascular permeability response. Forskolin greatly potentiated the footpad edema induced with carrageenin in rats. Intravenous administration of the enzyme bromelain, which reduces plasma kininogen levels inhibited the footpad edema induced with carrageenin or with a mixture of carrageenin and forskolin. Parenteral administration of a prostaglandin synthetase inhibitor, indomethacin, suppressed the footpad edema induced with carrageenin but did not inhibit the footpad edema induced with a mixture of carrageenin and forskolin. These results suggest that both bradykinin and prostaglandins may be essential for the development of carrageenin-induced footpad edema and that bradykinin may play an important role in the potentiative effect of forskolin on footpad edema induced with carrageenin in rats.

Derivatives of forskolin have been tested for their ability to stimulate membrane adenylate cyclase from rat brain and heart, as well as cyclic AMP-generation in guinea pig brain vesicular preparations, a model system for intact cells. Derivatives at the 6β- or 7β-hydroxy functions retain activity, but none have greater activity than that of forskolin. Reduction of the 11-keto function affords an active 11β-hydroxy derivative and an inactive 11α-hydroxy derivative. Reduction of the 14,15-vinyl (α) substituent reduces activity, while epoxidation abolishes activity. Derivatization of the 9α-hydroxy function results in a marked reduction in activity, further emphasizing the importance of the α-aspect of molecule. However, the 1α,6α-diacetyl derivative does retain activity. None of the inactive derivatives which include the 14,15-epoxy-, the 1,9-dideoxy- and the 1,9-diketo-derivatives antagonize the stimulatory effects of forskolin.

Calcium stimulates adenylate cyclase activity in rat cerebral cortical membranes with either ATP or AppNHp as substrate. In contrast, isoproterenol stimulates the cerebral cortical enzyme with ATP as substrate but not with AppNHp as substrate unless exogenous GTP is added. In rat striatal membranes, calcium or dopamine stimulate adenylate cyclase activity with ATP as substrate, but not with AppNHp as substrate. GTP restores the dopamine but not the calcium response. The inhibitory guanine nucleotide GDP-βS antagonizes dopamine and GppNHp stimulation of the brain adenylate cyclases, but not stimulation by calcium of either rat cerebral cortical or striatal enzymes. Results indicate that GTP is not requisite to calcium-calmodulin activation of adenylate cyclases in brain membranes. In addition, calcium-calmodulin cannot activate striatal adenylate cyclase with a nonphosphorylating nucleotide, AppNHp, as substrate.

Membranes from rat cerebral cortex and striatum contain a relatively large number of high affinity binding sites for [^{3}H]2',5'-dideoxyadenosine, [^{3}H]adenine arabinoside, and [^{3}H]adenosine. The binding of [^{3}H]2',5'-dideoxyadenosine and [^{3}H]adenine arabinoside is virtually unaffected by relatively specific agonists and antagonists for adenosine receptors, such as 2-chloroadenosine, N^6-phenylisopropyladenosine or theophylline. Binding of [^{3}H]adenosine is partially blocked by such receptor ligands. The specific binding of all three ligands is antagonized by a variety of adenosine analogs which inhibit adenylate cyclase by interaction with the so-called P-site associated with this enzyme. However, potencies of adenosine analogs as P-site inhibitors of adenylate cyclase and as antagonists of binding do not correlate well. 5'-Methylthioadenosine has high potency and efficacy versus binding of [^{3}H]2',5'-dideoxyadenosine, but has virtually no effect on activity of adenylate cyclase. 2-Fluoroadenosine is less potent than adenosine as an antagonist of specific binding of [^{3}H]2',5'-dideoxyadenosine, while 2-fluoro-derivatives of adenosine, adenine arabinoside and adenine xylofuranoside are more potent than the parent compounds as P-site inhibitors. The significane of the binding sites for [^{3}H]2',5'-dideoxyadenosine remains unclear, but their presence complicates the use of [^{3}H] adenosine and certain analogs as ligands for adenosine membrane sites associated with adenylate cyclase.

Various antidepressant drugs (monoamine oxidase inhibitors and tricyclics) enhance norepinephrine availability and lead to adaptive changes in brain noradrenergic systems, namely, decreases in the number of beta receptors and in the responsiveness of adenylate cyclase to norepinephrine stimulation. After 21 days of 1 mg/kg/day of treatment with clorgyline, an A type selective MAO inhibitor, but not after 3 days, there is an increase in norepinephrine release from rat brain microsacs in response to 43 mM KCl stimulation. Microsacs prepared from 21-day clorgyline-treated animals also show a marked decrease in the inhibition of norepinephrine release caused by the α_2 selective agonist clonidine. These functional changes in norepinephrine release mechanism are accompaued by a 53% reduction in brainstem α_2 receptor density as measured by [^{3}H]clonidine binding. At the same time, despite findings of a large decrease in beta receptor number as determined by [^{3}H]dihydralprenolol binding data, no significant decrease in responses of cyclic AMP systems to norepinephrine stimulation is observed. Decreases in the cyclic AMP response are observed by day 35 of clorgyline treatment. The results provide direct physiological support for a change in the norepinephrine release mechanism and an effect on autoreceptors specifically, preceding post-synaptic adaptive changes in the instance of one antidepressant, clorgyline. Difficulties in observing such changes with other antidepressants may result from the multiple nature of alpha adrenergic receptors especially as measured by radioactive ligand techniques; the lack of a direct relationship between physiological changes and receptors as measured by radioligand techniques; the large doses of monoamine oxidase inhibitors used in some studies and the possible multiplicity of antidepressant molecular mechanisms.

Bilateral ischemia induced by occlusion of the carotid arteries for 5 min causes a selective degeneration of CA 1 neurons of the hippocampus of Mongolian gerbils. Degeneration is complete in 14 days. After one week, basal levels of radioactive cyclic AMP in slices from the CA 1 region of hippocampus are greatly reduced as are the absolute magnitude of accumulations of cyclic AMP elicited by norepinephrine, 2-chloroadenosine and histamine. At 2 and 4 weeks, basal values have nearly attained control values and the response to 2-chloroadenosine is fully restored. The response of cyclic AMP-generating systems to norepinephrine is now significantly greater than in control, while the response to histamine remains reduced in magnitude. The ischemia has no effect on radioactive cyclic AMP levels or responses in slices from cerebral cortex. Histamine levels after ischemia were significantly increased above control in the CA 1 region. Basal and histamine-sensitive adenylate cyclase activity were not significantly changed. However, there was an inverse correlation between histamine levels and adenylate cyclase activity.

Adenosine analogs activate adenylate cyclase in rat striatal membranes, elicit accumulations of cyclic AMP in striatal slices and inhibit binding of $[^3H]N^6$-cyclohexyladenosine to striatal membranes. Three adenosine receptors appear to be involved. The A_2-receptor which activates adenylate cyclase in membranes appears to be a much higher affinity receptor than that which stimulates accumulation of cyclic AMP in slices, suggesting that the limbic system contains both the usual low affinity A_2-receptor system whose coupling is lost in membranes, and a high affinity A_2-receptor system whose coupling to adenylate cyclase is maintained in membranes. It would follow that the high affinity system must contribute only marginally to total accumulations of cyclic AMP in striatal slices. The affinity of N^6-substituted adenosines for both membrane and slice A_2-receptor systems in striatum is very low, so that binding of $[^3H]$ cyclohexyladenosine to A_2-receptors remains insignificant and only binding at high affinity A_1-receptors is measured.

Receptors for Neurotransmitters and Drugs in the Central Nervous System

Previous studies from this laboratory have demonstrated that recognition sites for benzodiazepines are functionally linked to receptors for the major inhibitory neurotransmitter, GABA, and a chloride ionophore. The functional interaction of these three sites as part of a "supramolecular complex" has been useful in explaining the actions of compounds such as barbiturates and pyrazolopyridines, which share common pharmacologic actions with benzodiazepines but do not bind to benzodiazepine recognition sites. We have also previously demonstrated that certain C-3 substituted β-carbolines, which also bind to benzodiazepine receptors with high affinity, antagonize both the anxiolytic and anticonvulsant actions of benzodiazepines such as diazepam. The availability of tritiated, C-3 substituted β-carbolines with high specific radioactivity permitted us to compare the regulatory properties of benzodiazepine receptors when occupied by functional "agonists" and "antagonists".

In contrast to the apparent increased affinity of such receptors for agonist benzodiazepines observed in the presence of GABA, barbiturates, pyrazolopyridines, or chloride ions there is no increase in apparent affinity for the benzodiazepine antagonist 3-carboethoxy-β-carboline (β-CCE). This finding was extended to another structurally unrelated benzodiazepine antagonist, CGS-8216 (a pyrazoloquinolinone), suggesting the lack of regulation of benzodiazepine receptor affinity in the presence of modulatory agents (such as GABA) may be a common property of a variety of benzodiazepine receptor antagonists.

These observations have led to the development of a rapid, sensitive *in vitro* test which differentiates benzodiazepine-like compounds from antagonists. This screening procedure has proven to have excellent predictive value by *post hoc* analysis.

Studies to determine the molecular target size by radiation inactivation of benzodiazepine receptors when occupied by radiolabelled agonists and antagonists has shown the target size to be virtually identical, approximately 60,000 daltons.

Fundamental differences in the regulatory properties of the benzodiazepine receptor complex when occupied by an agonist or antagonist also prompted studies using an irreversible benzodiazepine, irazepine. Incubation of cerebellar membranes with irazepine followed by ligand binding studies using either [^{3}H]diazepam or β-CCE resulted in a loss of [^{3}H]diazepam binding sites (with a small accompanying change in apparent affinity) while an affinity change (with little or no change in site number) was observed using [^{3}H]β-CCE as a radioligand. This observation, taken together with molecular target size and kinetic studies, suggests that benzodiazepine agonists and antagonists bind to separate subsites or "domains" on the benzodiazepine receptor. The presence of domains on a protein with a molecular size of approximately 60,000 daltons (the "benzodiazepine receptor") for agonists and antagonists would explain the lack of enhancement of apparent receptor affinity by (e.g.) GABA and barbiturates in the presence of an antagonist, and is also consonant with kinetic studies suggesting agonists and antagonists bind to the same macromolecular entity. Similar results have recently been observed using a chemically unrelated benzodiazepine antagonist, Ro 15-1788, further supporting the domain concept.

The most extensively studied β-carbolines with benzodiazepine antagonist properties, β-CCE and 3-hydroxymethyl-β-carboline (3-HMC), do not appear to have intrinsic convulsant activity. However, the methyl analog of β-CCE (3-carbomethoxy-β-carboline, β-CCM) has recently been demonstrated to produce motor seizures in mice. These seizures could be effectively antagonized by diazepam, as well as the benzodiazepine antagonists Ro 15-1788 and CGS-8216, suggesting that occupation of either an agonist or antagonist domain may be sufficient to interrupt or prevent these seizures. Marked differences in susceptibility to the convulsant actions have been observed between strains of mice. The molecular bases of this strain difference is currently being investigated.

3-HMC has been previously demonstrated to antagonize the anxiolytic and anticonvulsant actions of diazepam. We have used this compound as a tool to investigate the role of the benzodiazepine receptor in the sedative/hypnotic actions of benzodiazepines. Administration of 3-HMC at doses (7.5 mg/kg, i.p.) which do not affect basal sleep parameters (defined electroencephalographically) in rats significantly antagonized the hypnotic actions of flurazepam, reflected in a significant reversal of flurazepam-elicited decreases in sleep latency and increases in total sleep time. Furthermore, slightly higher doses of 3-HMC elicited a significant increase in sleep latency and decreased total sleep time over a two hour recording period. These observations are the first definitive demonstration that the benzodiazepine receptor mediates the hypnotic actions of the benzodiazepines, and also strongly suggests the benzodiazepine receptor is involved in the physiologic control of sleep. These alterations in sleep patterns of the rat were not accompanied by significant changes in motor activity. In contrast to analeptics such as caffeine and amphetamine which elicit similar changes in EEG defined sleep but invariably also cause large increases in motor activity, 3-HMC appears to possess only the somnolytic action. These observations suggest that 3-HMC or a related compound could be potentially useful as a therapeutic agent in disease states associated with excess sleepiness.

Pilot studies are now in progress to examine whether some benzodiazepine antagonists (such as β-CCE) have anxiety producing actions (anxiogenic) in primates. Preliminary results have shown administration of β-CCE to be associated with rapid, marked elevations in plasma cortisol, as well as dramatic increases in mean arterial blood pressure and pulse rate. Pretreatment of monkeys with diazepam or Ro 15-1788 effectively antagonized these somatic manifestations of anxiety.

Previous studies have shown that administration of a reversible inhibitor of adenosine deaminase (EHNA; 9-(erythrohydroxynonyl adenine) results in a profound reduction in motor activity (a behavioral sedation) which is temporally paralled by an inhibition of brain adenosine deaminase activity. However, electroencephalographic measurement of these "behaviorally sedated" rats demonstrated significant reductions in total sleep time and dramatic increases in sleep latency. This paradoxical behavior has been termed "quiescent waking".

Administration of L-phenylisopropyladenosine (L-PIA), a potent analog of adenosine (which binds to adenosine A_1 receptors with an affinity of less than 10 nM) resulted in a long lasting behavioral sedation. However, EEG changes appear similar to those produced by EHNA. These observations suggest that although adenosine is behaviorally sedating, the electroencephalographic patterns are reminiscent of a behavioral activation. It had been proposed that the sedation elicited by adenosine and adenosine derivatives may be due to the hypotensive actions of these compounds. Indeed, administration of as little as 0.1 mg/kg of L-PIA elicites a profound reduction in the blood pressure of anaesthetized rats. However, administration of 8-p-sulfophenyltheophylline, an adenosine antagonist which does not cross the blood-brain barrier, resulted in a significant reversal of the hypotension produced by L-PIA, but does not reverse the behavioral sedation. These observations provide strong evidence that the sedation produced by L-PIA (and EHNA) is centrally rather than peripherally mediated.

Neurochemical studies suggest that a circadian rhythm in the number of adenosine A_1-receptors occur in rat hippocampus and cortex, but not cerebellum. Studies are currently in progress to further investigate the physiological significance of these changes with regard to altered sleep patterns in rats.

Previous studies have demonstrated that $[^3H]$imipramine labels a "serotonin transporter" (recognition site + transport protein) in brain and platelet of both human and rat. The number of these sites is profoundly reduced in platelets of depressed patients, yet does not return to "normal" values following clinical improvement of depression, suggesting this parameter could be an important marker for depressive illness. The demonstration of a strong concordance of $[^3H]$imipramine binding to platelets from monozygotic (identical) twins further strengthens the contention that this parameter may be a useful "biological marker" in depression. Studies are now in progress to solubilize the serotonin transporter and separate a recognition site for serotonin (and tricyclic antidepressants) from a transport protein, with the ultimate goal of reconstitution of this system in artificial membranes.

High affinity, saturable and stereospecific recognition sites for amphetamine (and related phenethylamines) has been demonstrated in mammalian brain. The highest densities of these sites are in hypothalamus and striatum, yet pharmacologic characterization of these sites suggest it is not related to recognition sites for any previously described neurotransmitter. Furthermore, a highly significant ($p < 0.01$; $r = 0.97$) correlation has been demonstrated between the potencies of a series of phenethylamine derivatives to displace [^{3}H]amphetamine from hypothalamic membranes and their potencies as anorectic agents. A similar correlation was not obtained for the motor stimulant properties of these compounds, suggesting these sites may be a locus for the appetite suppressing properties of amphetamine and related compounds.

A stereospecific inhibition of [^{3}H]phencyclidine (^{3}H-PCP) binding to brain slices has been observed using an enantiomeric pair of PCP analogs (phenylcyclohexyl-3-methylpiperidine; PCMP). The differences in potency observed for this enantiomeric pair is in good agreement with the behavioral and electrophysiological differences already reported by this laboratory.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 31100-17 LBC

PERIOD COVERED
October 1, 1981 to September 20, 1982

TITLE OF PROJECT (80 characters or less)

Pharmacologically Active Compounds from Amphibians and Other Natural Sources

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2.9	0.6	2.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A total of over one hundred and fifty pharmacologically active alkaloids have been characterized in skin extracts of some fifty species of dendrobatid frogs. The batrachotoxins are steroid alkaloids which by interaction with a specific site prevent inactivation of voltage-dependent sodium channels in nerve and muscle. A radioactive batrachotoxin analog is a useful ligand for this site. Binding is antagonized competitively by local anesthetics. The histrionicotoxins are spiropiperidine alkaloids. The pumiliotoxin-C class are decahydroquinolines. The gephyrotoxins are indolizidines and perhydrobenzoindolizidines. These rather non-toxic alkaloids interact with site(s) on chemosensitive (acetylcholine, glutamate) ionic channels, thereby enhancing conversion to a non-conducting state. The pumiliotoxin-A class are 6-alkylidene-indolizidines which facilitate and then block calcium-dependent excitation-secretion and excitation-contraction. Prolongation of muscle twitches appears to be related to a specific inhibition of calcium-dependent ATPase of sarcoplasmic reticulum. Pumiliotoxin-B has no effect on other ATPases including the calmodulin-dependent calcium-ATPase of erythrocyte. Simpler analogs are, however, general inhibitors of many ATPases.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 31101-14 LBC

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Pharmacodynamic Amines and Enzymes Involved in Their Metabolism

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2.0	1.2	0.8

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☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The chemistry, biochemistry and pharmacology of biogenic amines, their amino acid precursors and metabolic products has been investigated. The areas of specific interest are: 1) the immunohistochemical localization of catechol-O-methyltransferase (COMT) in malignant and normal tissues of human and rodent. 2) The effect of the substitution of fluorine on the aromatic ring of biogenic amines, their amino acid precursors, and various alpha and beta-adrenergic antagonists. 3) The mechanism of toxicity and leukocytopoiesis of 2-fluorohistidine in normal and leukemic mice. 4) The characterization of the tryptophan-deficient rat. 5) The morphological characterization of vesicular bodies prepared from rodent brain by microphotometric birefringence "real time" microscopy. 6) The application of F19 nuclear magnetic resonance spectroscopy to the uptake release, metabolism and storage of 6-fluorodopamine in vesicular preparations from rodent striatum.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 31102-11 LBC

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

The Role of Cyclic Nucleotides in the Nervous System

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Forskolin, a diterpene, activates adenylate cyclase in broken cell preparations as well as in intact tissues. This activation does not require the guanine nucleotide regulatory subunit of the enzyme and probably occurs via an interaction with the catalytic subunit or an associated protein. Activation of adenylate cyclase by forskolin results in marked increases in levels of intracellular cyclic AMP in a variety of eukaryotic cells. Low concentrations of forskolin which alone elicit small increases in intracellular cyclic AMP greatly potentiate hormonal activation of adenylate cyclase in a number of intact cells. Forskolin elicits cellular responses which have been proposed to be dependent on cyclic AMP as a second messenger. Forskolin, thus, provides an invaluable tool for the investigation of the role of cyclic AMP both through its direct activation of adenylate cyclase and through its ability to potentiate activation of adenylate cyclase by biogenic amines, peptides, prostaglandins and adenosine. Activation of adenylate cyclase by calmodulin appears independent of guanyl nucleotides but may be influenced by membrane phosphorylation and is potentiated by forskolin. Striatum appears to contain three classes of adenosine receptors, two of which are stimulatory to adenylate cyclase.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 31103-04 LBC

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Receptors for Neurotransmitters and Drugs in the Central Nervous System

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Phil Skolnick	Pharmacologist	LBC	NIADDK
Other:	Margaret M. Schweri	Staff Fellow	LBC	NIADDK
	Evan F. Williams	Visiting Fellow	LBC	NIADDK
	Eberhard Kutter	Guest Worker	LBC	NIADDK

COOPERATING UNITS (if any) K. Rice, M. Rafferty, LC, NIADDK; E. Kempner, LPB, NIADDK; S. Paul M. Rehavi, NB, NIMH; P. Marangos, J. Patel, CPB, NIMH; W. Mendelson, K. Berman, APB, NIMH; J. Cook, M. Cain, F. Guzman, U. WI, Milwaukee; J. Crawley, E.I. DuPont; A. Lippa, D. Clody, Lederle Labs; O. Saiko, K. Irmscher, E. Merck.

LAB/BRANCH
Laboratory of Bioorganic Chemistry

SECTION
Section on Pharmacodynamics

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland, 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.5	3.0	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

High affinity, stereospecific recognition sites (receptors) for putative neurotransmitters, neuromodulators, and many psychotherapeutic agents have been identified in the mammalian central nervous system. It is currently believed that the interaction of a neurotransmitter, neuromodulator, or psychotherapeutic agent with these sites initiates a series of events resulting in either a physiologic/behavioral response (in the case of neurotransmitters and neuromodulators) or a pharmacologic action (in the case of a psychotherapeutic agent). Furthermore, the high affinity, stereoselective binding of psychotherapeutic agents to brain also suggests that previously undescribed endogenous modulators, which physiologically mimick (or antagonize) the actions of these agents may be present in brain. Several receptor systems are currently under study, including: a) benzodiazepine receptors (believed to play a role in the pathophysiology of anxiety, seizure disorders, musculoskeletal disorders, and sleep disorders; b) adenosine receptors (believed to be involved in sleep/wakefulness); c) receptors for tricyclic antidepressants; d) receptors for central stimulants (e.g., amphetamine); e) receptors for hallucinogens (e.g. phencyclidine).

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 31104-14 LBC

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Enzymatic Oxidation of Drugs to Toxic and Carcinogenic Metabolites

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. M. Jerina	Chief, Sect. on Oxidation Mech.	LBC	NIADDK
OTHER:	H. Yagi	Visiting Scientist	LBC	NIADDK
	D. R. Thakker	Senior Staff Fellow	LBC	NIADDK
	R. A. Halpin	Staff Fellow	LBC	NIADDK
	D. P. Michaud	Staff Fellow	LBC	NIADDK
	P. J. van Bladeren	Visiting Fellow	LBC	NIADDK
	K. P. Vyas	Visiting Fellow	LBC	NIADDK
	J. M. Sayer	Guest Worker	LBC	NIADDK
	T. Shibata	Guest Worker	LBC	NIADDK
	M. Nordqvist	Guest Worker	LBC	NIADDK

COOPERATING UNITS (if any)
Drs. A. Conney, W. Levin and A. Wood, Hoffmann-La Roche, NJ; Dr. D. Whalen, Univ. of Maryland at Catonsville, MD; Dr. R. Lehr, Univ. Oklahoma, OK; Dr. D. R. Boyd, Queen's Univ., Belfast, N. Ireland.

LAB/BRANCH
Laboratory of Bioorganic Chemistry

SECTION
Section on Oxidation Mechanisms

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
8	7	1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The primary goal of this project has been the elucidation of the structure of reactive metabolites which are responsible for the carcinogenic, cytotoxic, and mutagenic activity of benzo[a]pyrene and other polycyclic hydrocarbons. The approach taken consists of: i) synthesis of primary oxidative metabolites as well as selected secondary oxidative metabolites, ii) study of the metabolism of these hydrocarbons with liver microsomes, as well as with purified and reconstituted cytochrome P-450 systems with and without epoxide hydrolase, iii) tests for inherent mutagenicity of the synthetic metabolites toward bacterial and mammalian cells, iv) elucidation of the roles of the cytochrome P-450 system and epoxide hydrolase in potentiating or obliterating the mutagenicity of these metabolites, v) determination of the carcinogenic activity of these compounds, vi) determination of the rate and nature of the products formed when reactive metabolites such as arene oxides and diol epoxides react with biopolymers and less complex model compounds. A general theory of hydrocarbon-induced carcinogenesis has been formulated and its predictions are being tested with synthetic potential metabolites of several hydrocarbons. Based on the presently available data, the bay region theory has excellent predictive value.

ANNUAL REPORT OF THE

LABORATORY OF MOLECULAR BIOLOGY

The Laboratory of Molecular Biology has as its principal goal the understanding of biological processes at the molecular level. The research areas under investigation include the structures and chemical properties of biologically important materials. These involve studies of the organization of DNA and proteins in chromatin, investigations of enzyme and immunoglobulin structures by x-ray diffraction, investigation of polynucleotide chemistry and structure by synthetic and spectroscopic methods, studies of polynucleotide structure by fiber diffraction methods, and theoretical studies on the mechanism of energy conversion in biology, muscle contraction, the statistical thermodynamics of polynucleotide - complementary monomer interactions, ion transport and biochemical kinetics. In addition, other investigations are concerned directly with biological processes. These include the mechanism of transformation by SV40, the calorimetric detection of viral infections, genetic recombination, and DNA synthesis. Important advances have been made in these areas during this year.

Chromatin Structure and Function

We have continued studies of chromatin structure, of the structural differences between transcriptionally active and inactive chromatin, and the possible role of left-handed Z DNA in chromatin structure. We have shown that a DNA segment 115 base pairs long, near the 5' end of the chicken adult β-globin gene, can be released in high yield from red blood cell nuclei by digestion with MspI endonuclease. The release occurs only in nuclei transcribing the globin region and shows that a special nucleosome-free region, probably related to gene expression, exists in such cells. We have also studied the effects of high mobility group proteins on folding of chromatin, and the effect of chemically blocking the large groove of DNA on nucleosome formation. We have also shown that the presence of left-handed Z DNA has a major effect on the ability of DNA to form nucleosomes.

Three-Dimensional Structure of Proteins of the Immune System

The refinement of the 2.7 A x-ray structure of the phosphocholine-binding mouse IgAk immunoglobulin Fab has been continued. Further improvement in the quality of the model is being achieved by the inclusion of solvent molecules. An examination of the van der Waals surface of the combining site shows the very good fit of bound phosphocholine in the binding site.

The crystal structure of the galactan-binding mouse Fab, J539, has now been solved. The model is undergoing refinement by means of the program PROLSQ and examination of our interactive graphics system using the program BILDER. The current resolution of 3.5 A is being extended to 2.6 A. The "elbow bend" is slightly different from that in the McPC653 model but the variable and constant domains have essentially the same backbone structures, taken pair by pair, in the two models.

Three-Dimensional Structures of Enzymes

The earlier model for the aspartic protease from Rhizopus chinensis at 2.5 A resolution is now being refined using data to 1.8 A. The higher resolution is assisting in the deduction of the amino acid sequence which remains chemically unknown.

Crystals of bovine heart creatine kinase MM isozyme have been obtained and characterized. The crystals diffract well to at least 3.0 A resolution and appear to be suitable for a full three-dimensional study of the enzyme.

Collaborative research with A. Wlodawer of the National Bureau of Standards, Gaithersburg, has resulted in a refinement of the x-ray and neutron structures of the digestive enzyme ribonuclease by a new joint refinement procedure. This work is continuing now with the examination of the complex between ribonuclease and an analogue of an activated substrate, uridine vanadate.

Statistical Thermodynamics of Protein and Polynucleotide Systems

Three different topics have been studied under this project: (a) We have developed a new improved procedure for generating circular ring configurations (of the freely-jointed model of DNA) and we have estimated the ring-to-chain ratios of radii of gyration and sedimentation coefficients at infinite molecular weight and compared the results with those obtained by the so-called ε-method; (b) We have proved that the McGhee-von Hippel theory is exact from a statistical mechanical view point, using the matrix method, and we have devised a new type of isotherm plot that can establish the mechanism of binding in cases not amenable to other existing plots; and (c) We have used the concept of once-visited sites in random walk theory to study isotope exchange experiments in biopolymers that undergo a helix-coil transition, and in F-actin or microtubules that undergo head-to-tail polymerization.

Energy Conversion in Biology, Especially Muscle Contraction

Three different topics have been studied under this project: (a) on the difficult problem of the effect of nearest-neighbor interactions on the steady-state kinetics of enzyme molecules in large lattices, an exact result derived in the last year is the calculation of the Onsager linear coefficient L in the flux-force relation for steady states near equilibrium of a one-dimensional lattice of two-state enzyme molecules; (b) a very complete theoretical analysis has been carried out of the ways in which microtubules and actin filaments can do mechanical work by lengthening, shortening, or treadmilling at constant length with a load attached; and (c) we have developed a cooperative model of the sarcoplasmic reticulum Ca-ATPase that fits equilibrium data on the binding of Ca^{++} and H^{+} very well and we are in the process of attempting to fit steady-state ATPase data by the same model.

Calorimetric Detection of Virus Infection of Cells Grown in Tissue Culture

Monkey kidney cells (Vero) growing in tissue culture in 35 mm Petri dishes have been observed in a calorimeter possessing long term stability of 0.5 microwatts. Cells in monolayer culture in maintainance medium were found to give a constant level of heat production that was stable for over five days. This base line characteristic of living cells was established to be exothermic relative to the identical instrumental base line exhibited by medium alone and cells killed by exposure to either methanol, formaldehyde or γ-radiation. Infection of living Vero cells by an attenuated strain of poliovirus exhibited a broad inverse sigmoidal transition between these two regimes. Microscopic examination showed the fibroblasts to become progressively rounded-up and detached concurrent with the decrease in heat production. The characteristic time parameters of the decreased heat production were found to be inversely proportional to the viral inoculum over a 10^7-fold range of initial virus concentration, demonstrating that calorimetry may be used for the assay of viral titre. Inadvertent bacterial contamination of the sample is rapidly detected by calorimetry.

Thermodynamics of Protein-Ligand and Macromolecular Assembly Complexes

A detailed study of the effects upon the thermodynamic stability of both human and bovine serum albumin resulting from: (a) the method of purification, (b) incubation at 60°C, (c) ligand binding at the fatty acid and aromatic binding site(s) has been carried out by differential scanning calorimetry. Enthalpic and entropic contributions to the protein stability have been evaluated.

The major structural protein of the bacteriophage T4 capsid, gp 23, self-assembles into various microtubular "polyhead" structures. The thermodynamic parameters associated with the conversion of the "expanded" lattice polyhead to denatured monomeric cleaved protein, gp 23*, has been determined by differential scanning calorimetry.

Poly 2-aminodeoxyadenylic Acid

We have found previously that introduction of a 2-NH_2 group into poly r(A) permits formation of three hydrogen bands of poly r(U) and causes the A•U complexes to resemble in some respects the more familiar G•C pairs. We are now investigating 2-aminoadenine derivatives in the deoxypolynucleotide area in order to study conformations not accessible to the less flexible ribopolynucleotides. Our recent synthesis and study of poly 2-aminodeoxyadenylic acid is directly relevant to the remarkable discovery of Khudyakov et al. that DNA of cyanophage S-2L has all of its adenine residues replaced by 2-aminoadenine. We find that spectroscopic and physical properties of the new polymer can account for some surprising properties of the unusual DNA of cyanophage S-2L.

Complex of Poly(X) with Poly(U). The First Hetero Four-Stranded Helix

We have proposed a novel structural model for the complex formed by polyxanthylic acid and polyuridylic acid and have obtained experimental evidence to support the model. The structure is a four-stranded helix, X_2U_2, where like residues are monoionized, and alkali metal ions occupy size-specific binding sites in the axial channel of the helix. These metal ions partially screen electrostatic repulsion of the two X residues.

G•C Complex Formation in Template-Dependent Oligo(G) Synthesis

We have made an infrared study of the template-dependent synthesis of oligo(G) on a poly(C) template (Inoue and Orgel, 1982) in order to determine which, if any, complexes are present and what role the template plays. At pH 8 under synthetic conditions (Mg^{++} present), there is initially very little complex present. This small amount is two-stranded G•C helix. The rate of complex formation is slow and parallels the chemical synthesis. Most of the G present in the complex at any time has already been polymerized, in contrast to preponderance of monomer on the template under non-synthetic conditions (no Mg^{++}). Synthesis proceeds primarily at growing points at the ends of the G•C helical regions.

Structural Studies on Polynucleotides

The aim of this project is to obtain structural information on polynucleotides and other polymers and to provide insights into the factors which control structure, principally through x-ray fiber diffraction and model building studies. Current areas include: (1) correlation of the fiber structures of poly(rA)•poly(dT) with solution and other properties of non-diffraction techniques; (2) a reexamination of poly(dA)•poly(dT) by fiber diffraction techniques; (3) the test of a possible artefact in our earlier wet DNA fiber studies.

Mammalian Enzymes Involved in Repair or Replication of DNA

Currently we are continuing to examine the tendency for single-strand breaks in DNA to assume specific localizations within nucleosomes in chromatin, and the ability of a eukaryotic DNA ligase to seal such single-strand breaks while in chromatin.

Genetics and Structure of the Oncogenic Virus, SV40

Analysis of the physical chemistry of linear DNA containing replication loops has demonstrated that the loops are remarkably unstable and are extruded as double-stranded DNA at a significant rate even at temperatures 50° and more below the T_m. The rate limiting step in this extrusion is the rate of end melt and/or nucleation of the nascent strands, not the rate of branch migration. This unusual feature provides a general method for the isolation of origins of DNA replication.

Novel Recombination Systems

Two genes in *E. coli* affect specific aspects of transposition. The *sap* gene is required for the transposition of an *IS1* or *IS5* to a site in the Bgl operon which turns on the operon. Mutations in the *rho* gene affect the ability of the cells to carry out intra-chromosomal transposition but not interchromosomal transposition.

Study of Functions Involved in Genetic Recombination

We have studied the control of DNA supercoiling in *E. coli*, and have found several regulatory interactions. Topoisomerase I stimulates the synthesis of both subunits of DNA gyrase, while the gyrase B protein represses the synthesis of gyrase A protein. The HimA protein, a known regulatory effector, stimulates gyrase A (but not B) protein synthesis.

We have also sequenced the region around the start of the gyrB gene, and identified the beginning of the messenger RNA and of the protein chain.

Studies on the Mechanism of Genetic Recombination

The major object of this project is to uncover enzymatic steps involved in various genetic rearrangement reactions and study the mechanism of action of the enzymes involved. The reactions studied include: (1) the site specific recombination of bacteriophage lambda, (2) resolution of the intermediate of general genetic recombination, and (3) transposition-replication reaction of bacteriophage Mu. The most recent developments include the finding that endonuclease VII coded by bacteriophage T4 can resolve the Holliday structure, a typical general recombination intermediate, at high efficiency. Endonuclease VII and DNA ligase can cut and reseal the Holliday structure to restore normal DNA structure. This is the first example of the enzymatic step involved in this process.

Replication of Colicin El Plasmid DNA

In vitro replication of ColEl plasmid DNA depends solely on *E. coli* proteins. RNA polymerase synthesizes a transcript that is processed by RNase H and used as a primer for DNA synthesis by DNA polymerase I. Transcripts that start 555 nucleotides upstream origin either form hybrid with the template DNA near the origin or pass the origin without forming the hybrid. The transcripts that form hybrid can be cleaved by RNase H and used as the primer for DNA synthesis. The mechanism of action of RNase H has been studied in detail. It appears that the enzyme recognizes the sequence or structure of RNA and cleave the primer transcript preferentially at the origin.

Primer formation was found to be inhibited by a RNA I that starts 447 nucleotides upstream of the origin and terminates near the site where the primer starts on the opposite strand. Whether or not the primer transcript form hybrid with the template DNA is determined by the secondary structure which the transcript forms. Interaction of RNA I with the transcript prevents formation of the required structure by the transcript and thus prevents primer formation. *In vivo* properties of the plasmid, such as the copy number and incompatibility, can be explained by inhibition of primer formation by RNA I. Importance of specific RNA secondary structures for primer formation is demonstrated also by the effect of substitution of GMP residues in the primer by IMP residues. The substitution changes the structure of RNA and thus inhibits primer formation. The nucleotide sequences of the regions that include the origin of DNA replication of plasmid RSF 1030 and p15A were determined and their sequence were compared with those of other ColEl related plasmids. Their sequences are quite similar, reflecting the similar mechanism they use for replication. But they also contain unique sequences that provide clues for understanding the mechanism of replication.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 33000-16 LMB

PERIOD COVERED
October 1, 1981 through Spetember 30, 1982

TITLE OF PROJECT (80 characters or less)
Study of Functions Involved in Genetic Recombination

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Martin Gellert,	Chief, Metabolic Enzymes Section	LMB NIADDK
OTHERS:	Anthony Maxwell	Visiting Fellow	LMB NIADDK
	Rolf Menzel	Staff Fellow	LMB NIADDK
	Kiyoshi Mizuuchi	Visiting Scientist	LMB NIADDK
	Mary O'Dea	Chemist	LMB NIADDK

COOPERATING UNITS (if any)
Prof. David Friedman, University of Michigan, Ann Arbor, Michigan

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
Section on Metabolic Enzymes

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 4	PROFESSIONAL: 3	OTHER: 1

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We have studied the control of DNA supercoiling in E. coli, and have found several regulatory interactions. Topoisomerase I stimulates the synthesis of both subunits of DNA gyrase, while the gyrase B protein represses the synthesis of gyrase A protein. The HimA protein, a known regulatory effector, stimulates gyrase A (but not B) protein synthesis.

We have also sequenced the region around the start of the gyrB gene, and identified the beginning of the messenger RNA and of the protein chain.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 33006-04 LMB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Studies on the Mechanism of Genetic Recombination.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	K. Mizuuchi	Visiting Scientist	LMB NIADDK
OTHERS:	M. Mizuuchi	Guest Worker	LMB NIADDK
	T. Adachi	Guest Worker	LMB NIADDK

COOPERATING UNITS (if any)
Robert Weisberg, NICHHD, NIH
Borries Kemper, University of Cologne, Cologne, W. Germany
John Hays, University of Maryland

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
Section on Metabolic Enzymes

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	1	1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The major object of this project is to uncover enzymatic steps involved in various genetic rearrangement reactions and study the mechanism of action of the enzymes involved. The reactions studied include: the site specific recombination of bacteriophage lambda, resolution of the intermediate of general genetic recombination, and transposition-replication reaction of bacteriophage Mu. The most recent developments include the finding that endonuclease VII coded by bacteriophage T4 can resolve the Holliday structure, a typical general recombination intermediate, at high efficiency. Endonuclease VII and DNA ligase can cut and reseal the Holliday structure to restore normal DNA structure. This is the first example of the enzymatic step involved in this process.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 33051-10 LMB

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Mammalian Enzymes Involved in Repair or Replication of DNA

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	S.B. Zimmerman	Research Chemist	LMB NIADDK
OTHER:	B.H. Pheiffer	Chemist	LMB NIADDK

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Molecular Biology

SECTION

Section on Physical Chemistry

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0.6	PROFESSIONAL: 0.1	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Currently we are continuing to examine the tendency for single-strand breaks in DNA to assume specific localizations within nucleosomes in chromatin, and the ability of a eukaryotic DNA ligase to seal such single-strand breaks while in chromatin.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 33054-07 LMB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Structural Studies on Polynucleotides

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	S.B. Zimmerman	Research Chemist	LMB NIADDK
OTHER:	B.H. Pheiffer	Chemist	LMB NIADDK

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
Section on Physical Chemistry

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.4	0.9	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The aim of this project is to obtain structural information on polynucleotides and other polymers and to provide insights into the factors which control structure, principally through X-ray fiber diffraction and model building studies. Current areas include (1) correlation of the fiber structures of poly(rA)·poly(dT) with solution and other properties by nondiffraction techniques; (2) a reexamination of poly(dA)·poly(dT) by fiber diffraction techniques, (3) the test of a possible artefact in our earlier wet DNA fiber studies.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 33056-02 LMB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Calorimetric Detection of Virus Infection of Cells Grown in Tissue Culture

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. D. Ross	Research Chemist	LMB NIADDK
OTHERS:	N. H. Woodruff, Jr.	Guest Worker	Fort Detrick, Md.

COOPERATING UNITS (if any)
U.S.A.M.R.I.I.D., Ft. Detrick, Frederick, Md.

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
Section on Physical Chemistry

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 1	PROFESSIONAL: 1	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Monkey kidney cells (Vero) growth in tissue culture in 35 mm Petri dishes have been observed in a calorimeter possessing long term stability of 0.5 microwatts. Cells in monolayer culture in maintainance medium were found to give a constant level of heat production that was stable for over five days. This base line characteristic of living cells was established to be exothermic relative to the identical instrumental base line exhibited by media alone and cells killed by exposure to either methanol, formaldehyde or γ-radiation. Infection of living Vero cells by an attenuated strain of poliovirus exhibited a broad inverse sigmoidal transition between these two regimes. Microscopic examination showed the fibroblasts to become progressively rounded-up and detached concurrent with the decrease in heat production. The characteristic time parameters of the decreased heat production were found to be inversely proportional to the viral inoculum over a 10^7-fold range of initial virus concentration, demonstrating that calorimetry may be used for the assay of viral titre. Inadvertant bacterial contamination of the sample is rapidly detected by calorimetry.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 34001-17 LMB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Chromatin Structure and Function

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. Felsenfeld	Chief, Sect. on Phys. Chem.	LMB NIADDK
OTHER:	M. Behe	Guest Worker	LMB NIADDK
	J. McGhee	Visiting Scientist	LMB NIADDK
	J. Nickol	Staff Fellow	LMB NIADDK
	D. Jackson	Chemist	LMB NIADDK
	G. Sandeen	Chemist	LMB NIADDK
	W. Wood	Staff Fellow	LMB NIADDK
	W. Komaiko	Research Associate	LMB NIADDK

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
Physical Chemistry Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
8	8	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We have continued studies of chromatin structure, of the structural differences between transcriptionally active and inactive chromatin, and the possible role of left-handed Z DNA in chromatin structure. We have shown that a DNA segment 115 base pairs long, near the 5' end of the chicken adult β globin gene, can be released in high yield from red blood cell nuclei by digestion with MspI endonuclease. The release occurs only in nuclei transcribing the globin region and shows that a special nucleosome-free region, probably related to gene expression, exists in such cells. We have also studied the effects of high mobility group proteins on folding of chromatin, and the effect of chemically blocking the large groove of DNA on nucleosome formation. We have also shown that the presence of left-handed Z DNA has a major effect on the ability of DNA to form nucleosomes.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 34002-18 LMB

PERIOD COVERED
October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)

Enzyme Structure

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. R. Davies	Chief, Sec. on Mol. Structure	LMB NIADDK
OTHER:	E. W. Silverton	Research Chemist	LMB NIADDK
	R. Bott	Staff Fellow	LMB NIADDK
	G. Gilliland	Staff Fellow	LMB NIADDK
	M. Miller	Visiting Fellow	LMB NIADDK

COOPERATING UNITS (if any)

Alexander Wlodawer, NBS; John Weinstein, NCI-LTB

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
Molecular Structure

INSTITUTE AND LOCATION
NIADD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.25	3.25	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

X-ray diffraction data to 1.8 A have been collected from crystals of the aspartic protease from Rhizopus chinensis. The higher resolution structure is under refinement. Work is continuing to improve the quality of crystals from mouse renin.

Crystals of creatine kinase have been obtained.

Crystals of plakalbumin have been obtained.

The neutron diffraction investigation of bovine ribonuclease A has been extended to 2.0 A and a joint X-ray/neutron structure for the enzyme has been refined.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 34003-14 LMB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Crystal Structure Investigation of Proteins of the Immune System

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. R. Davies	Chief, Sec. on Mol. Structure	LMB NIADDK
OTHER:	G. H. Cohen	Research Chemist	LMB NIADDK
	G. Gilliland	Staff Fellow	LMB NIADDK
	E. W. Silverton	Research Chemist	LMB NIADDK
	S. W. Suh	Visiting Fellow	LMB NIADDK

COOPERATING UNITS (if any)
D. Sachs, NCI-I; J. Coligan, NIAID-LIG; E. Kabat, NIADDK-IRP; M. Potter, NCI-I; J. Berzofsky, NCI-MET; B. Frangione, New York University Med. Center

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
Molecular Structure

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 3.75	PROFESSIONAL: 3.75	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Further refinement has been continued on McPC603 Fab, a murine phosphocholine specific myeloma protein.

The structure of the murine galactan-specific J539Fab has been solved and refinement has been commenced.

Attempts are underway to crystallize various histocompatibility antigens as well as a number of hybridoma immunoglobulin Fab fragments specific for lysozyme, myoglobin and certain oligosaccharides.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 35000-18 LMB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Chemical and Structural Investigations of Nucleic Acids and Related Substances

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. Todd Miles	Chief, Section on Organic Chemistry	LMB:NIADDK
OTHERS:	Frank B. Howard	Research Scientist	LMB:NIADDK
	Kunal B. Roy	Visiting Associate	LMB:NIADDK
	Eiko Nakagawa	Visiting Fellow	LMB:NIADDK
	Zu-kun Tan	Visiting Fellow	LMB:NIADDK

COOPERATING UNITS (if any)
Dr. Leslie Orgel, The Salk Institute, La Jolla, California

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
Organic Chemistry Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.5	4.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project has the long-range objective of studying the chemistry and structure of nucleotides, polynucleotides and nucleic acids and the application of these studies to the understanding of biochemical and biological processes. Topics of current interest include: (1) structure and reactivity of polynucleotides and the relation between the two; (2) stabilization of ordered forms of nucleic acids and polynucleotides; (3) spectroscopic studies of polynucleotides: infrared, ultraviolet, and circular dichroism; (4) design and synthesis of new polynucleotides having properties required for the foregoing studies.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 35050-11 LMB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Replication, Recombination and Repair of Microbial DNA

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Jun-ichi Tomizawa	Chief, Sec. on Mol. Genetics	LMB NIADDK
OTHER:	Tateo Itoh	Visiting Associate	LMB NIADDK
	Gerald Selzer	Staff Fellow	LMB NIADDK
	Tapan Som	Visiting Associate	LMB NIADDK
	Hisao Masukata	Visiting Associate	LMB NIADDK

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
Molecular Genetics Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 5	PROFESSIONAL: 5	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Studies on DNA replication of plasmid ColEl and its relatives have been continued. Initiation of plasmid DNA replication by RNA polymerase, ribonuclease H and DNA polymerase I have been studied. The mechanism of inhibition of primer formation by plasmid specific small RNA was elucidated. The nucleotide sequences of the regions that include the origin of DNA replication of plasmid RSF1030 and p15A were determined and the comparative studies on nucleotide sequences of ColEl related plasmids were carried out.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 36002-08 LMB

PERIOD COVERED
October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)

Novel Recombination Systems

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. L. Rosner	Research Biologist	LMB NIADDK
Other:	Atin R. Datta	Visiting Fellow	LMB NIADDK

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
Microbial Genetics

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Two genes in E. coli affect specific aspects of transposition. The sap gene is required for the transposition of an IS1 or IS5 to a site in the bgl operon which turns on the operon. Mutations in the rho gene affect the ability of the cells to carry out intra-chromosomal transposition but not inter-chromosomal transposition.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 36051-14 LMB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Genetics and Structure of the Oncogenic Virus, SV40.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Robert G. Martin, Chief, Section on Microbial Genetics	LMB NIADDK
OTHERS:	Maria Hadjopoulos, Visiting Scientist	LMB NIADDK
	Carlo Nobile, Visiting Fellow	LMB NIADDK
	Shaw S. Wang, Bio Lab Technician	LMB NIADDK
	Joanne Nickol, Staff Fellow	LMB NIADDK

COOPERATING UNITS (if any)
David Livingston, Sidney Farber Cancer Institute, Boston, MA
David Tenen, Sidney Farber Cancer Institute, Boston, MA

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
Microbial Genetics

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.5	3.5	0

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Nascently replicating strands of SV40 DNA have been isolated and cloned as an approach to the general problem of isolating mammalian origins of DNA replication. The analysis has demonstrated that rearrangement of the cloned DNA occurs with such high frequency that the general application of this method is not yet feasible. From the experiments, we have also found that DNA fork migration in bidirectionally replicating SV40 occurs at remarkably uniform rates. Of 33 clones analyzed, each SV40 fork had proceeded no more than 400 nucleotides further than the other and most were within 200 nucleotides of each other.

In other experiments, a deletion of 18 base pairs in the 27 base pair palindromic sequence at the SV40 replication origin has been isolated. A DNA fragment including this deletion was hybridized to the corresponding wild-type DNA fragment generating heteroduplex molecules in which the palindromic sequence is forced into the stem and loop form. A series of experiments have been performed with the heteroduplex molecules to study the function of DNA in the stem-loop form.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 36101-08 LMB

PERIOD COVERED
October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)

Energy conversion in biology, especially muscle contraction

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Terrell L. Hill	Chief, Section on Theoretical Molecular Biology, LMB, NIADDK

COOPERATING UNITS (if any)

Marc W. Kirschner	University of California, San Francisco
Giuseppe Inesi	University of Maryland, Baltimore

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
Theoretical Molecular Biology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Three different topics have been studied under this project: (a) on the difficult problem of the effect of nearest-neighbor interactions on the steady-state kinetics of enzyme molecules in large lattices, an exact result derived in the last year is the calculation of the Onsager linear coefficient L in the flux-force relation for steady states near equilibrium of a one-dimensional lattice of two-state enzyme molecules; (b) a very complete theoretical analysis has been carried out of the ways in which microtubules and actin filaments can do mechanical work by lengthening, shortening, or treadmilling at constant length with a load attached; and (c) we have developed a cooperative model of the sarcoplasmic reticulum Ca-ATPase that fits equilibrium data on the binding of Ca^{++} and H^+ very well and we are in the process of attempting to fit steady-state ATPase data by the same model.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 36102-11 LMB

PERIOD COVERED
October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)

Statistical thermodynamics of protein and polynucleotide systems

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Terrell L. Hill	Chief, Section on Theoretical Molecular Biology LMB, NIADDK	
OTHER:	Yi-der Chen	Research Chemist	LMB, NIADDK
	Takashi Tsuchiya	Visiting Associate	LMB, NIADDK

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
Theoretical Molecular Biology

INSTITUTE AND LOCATION
NIADDK, LMB, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Three different topics have been studied under this project: (a) we have developed a new and improved procedure for generating circular ring configurations (of the freely jointed model of DNA) and we have estimated the ring-to-chain ratios of radii of gyration and sedimentation coefficients at infinite molecular weight and compared the results with those obtained by the so-called ε-method; (b) we have proved that the McGhee-von Hippel theory is exact from a statistical mechanical view point, using the matrix method, and we have devised a new type of isotherm plot that can establish the mechanism of binding in cases not amenable to other existing plots; and (c) we have used the concept of once-visited sites in random walk theory to study isotope exchange experiments in biopolymers that undergo a helix-coil transition, and in F-actin or microtubules that undergo head-to-tail polymerization.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 36104-01 LMB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Thermodynamics of Protein-Ligand and Macromolecular Assembly Complexes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. D. Ross	Research Chemist	LMB NIADDK
OTHERS:	Andrew Shrake	Chemist	BB DBBP
	Alasdair C. Steven	Visiting Scientist	LPB NIADDK
	Lindsay W. Black	Professor	Univ. of Maryland Medical School

COOPERATING UNITS (if any)
BB DBBP
University of Maryland Medical School, Baltimore, Md. 21201

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
Section on Physical Chemistry

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 1.5	PROFESSIONAL: 1.5	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A detailed study of the effects upon the thermodynamic stability of both human and bovine serum albumin resulting from: a) the method of purification, b) incubation at 60°C, c) ligand binding at the fatty acid and aromatic binding site(s) has been carried out by differential scanning calorimetry. Enthalpic and entropic contributions to the protein stability have been evaluated.

The major structural protein of the bacteriophage T4 capsid, gp 23, self-assembles into various microtubular "polyhead" structures. The thermodynamic parameters associated with the conversion of the "expanded" lattice polyhead to denatured monomeric cleaved protein, gp 23*, has been determined by differential scanning calorimetry.

PHS-6040
(Rev. 2-81)

ANNUAL REPORT OF THE

ARTHRITIS AND RHEUMATISM BRANCH

Branch members have been honored in several ways. Dr. Steinberg received the Commendation Medal of DHHS for his work with systemic lupus erythematosus (SLE). Dr. Plotz, Finbloom and Magilavy shared the quadrennial Societe Francaise de Rhumatologie Prize for their work on the effect of antigen monosaccharides in determining the cellular mechanism removing an antigen-antibody complex from the circulation of rodents. Dr. Decker was named Plenary Lecturer at the XVth International Congress of Rheumatology in Paris; the lecture dealt with concepts of pathogenesis of rheumatoid arthritis and derived from Branch studies of leukapheresis.

Substantial progress was made in the studies of IgE interaction with cell surfaces, a useful model of antigen-induced, antibody mediated cellular reactions in general. The two regions of the α chain of the IgE receptor appeared to be mirrored in the finding that both of the epsilon chains of IgE interact with it. The appropriate ratios of crude lipids and detergents are critical in efforts to extract and purify the α and β chains of the receptor intact. The resulting purified, stable receptors can be incorporated in multiple copies in liposomes where their function can be more readily studied. Studies of the kinetics of IgE binding showed that it is not diffusion controlled. The IgE receptors on mast cells are the easiest to work with but those on other cells, for example, macrophages, may be physiologically critical; receptors on macrophages have low binding affinities but have been successfully demonstrated with artificially cross-linked IgE molecules (25) (Parenthetic numbers refer to individual project numbers, here for example Z01 AM 41025, where the project is described in more detail). Upon internalization of ligand IgE removes the receptor from the cell surface and it is not rapidly replaced. The evidence favors the view that the α chain of the receptor does not, in fact, penetrate the cell membrane (51).

Several strains of autoimmune mice are now under regular study with comparisons between strains allowing conclusions that were not earlier possible. Defective tolerance to bovine gamma globulin is typical of at least three strains, NZB, MRL-lpr, and BXSB. In the latter strain, only the males are tolerance defective and their autoimmune disease is accelerated over that of the females (20). Continuing efforts with hybridoma technology have resulted in an IgM-producing clone, derived from the B cells of an SLE patient and reacting with the dG.dC sequences of DNA (21). A diet enriched in fish oil containing eicosapentanoic acid greatly prolonged the life and reduced the proteinuria of NZB X NZW F1 mice; such a diet augments the production of pro-inflammatory prostaglandins, substances which have earlier been shown on direct injection to retard the model disease (22).

Cell walls from Group A and B streptococci (but not from Group D) given to rats as a single intraperitoneal injection are followed by polyarthritis which is remarkably persistent and destructive. Some rat strains are totally resistant to the arthritis not because the triggering antigen can be catabolized by one strain and not the other but because the antigen is distributed to the joints in one and not the other; this differential distribution may relate to differences in macrophage function (48).

Studies of the nature of human SLE continue to revolve about the large number of circulating B cells in patient blood which produce colonies on culture even though the corresponding T-cells do not help as in the normal (23). There was also interest in the natural killer (or NK) cells of the blood; they are reduced in number in the blood of SLE patients, perhaps due to autoantibody action, and do not function as vigorously as normal NK cells upon addition of interferon (52).

Branch clinical studies directed toward better management of patients with diffuse lupus nephritis (00, 40, & 56) cover many years and represent progressive advances. In sum oral cytotoxic drugs seemed to have little effect; intermittent, intravenous or combined cytotoxic drugs by mouth did provide some modest improvement; and, finally, we are embarked on a study of vigorous intravenous treatment for the active periods of the disease. Concurrent with much of this work has been intensive evaluation of the renal biopsies both before and after two years of a treatment program (38). Combining these two groups of studies has produced striking data of importance. Those patients with the lowest Chronicity Index, a scaled biopsy description, did well and those with the highest did badly irrespective of their assigned treatment. In the intermediate group of 14 patients, the cytotoxic drugs had a profound effect in preventing endstage renal failure ($p=0.0004$).

High doses of intravenous methylprednisolone (pulse) given as needed (53) and leukapheresis (54) are being studied in the management of rheumatoid arthritis. There are theoretical reasons for believing that pulse steroids and azathioprine might together suppress inflammation more thoroughly than would be expected by summing their separate effects; this is being tested.

With leukapheresis, analysis of the therapeutic response of further patients has made it clear that those patients with the lowest degrees of skin test reactivity and the poorest lymphocyte proliferative responses to antigens will do well on leukapheresis; they recover their T-cell responsiveness as the joint inflammation recedes. The findings have lead us to examine and characterize more thoroughly the synovial membrane lymphocytes; this is being done with arthroscopic biopsies.

Various other treatment programs are being used in the outpatient department on rheumatoid arthritis (49, 50, 57). We are a contributing clinic to the Cooperating Clinics for Systematic Studies of Rheumatic Diseases and these projects are done under their protocols.

Two other therapeutic studies are in progress. The first seeks to answer the question "Does plasmapheresis add to the benefit of immunosuppressive drugs in patients with Goodpasture's syndrome?" The Branch contribution is part of a multicenter study (59). The second is based on the observation that complicated and non-healing fractures can be induced to heal with pulsed electromagnetic field therapy (58). Since the treatment is non-invasive and apparently innoccuous, it was deemed appropriate to use it in the management of aseptic necrosis of bone, a complication which is, unfortunately, all too common in our SLE population; informed patients are being randomized to real and sham treatment.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 41000-13 ARB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Controlled study of cyclophosphamide and azathioprine in systemic lupus erythematosus with nephritis (CAP)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J.L. Decker	Chief, Arthritis & Rheumatism Br.	ARB NIADDK
OTHER:	P.H. Plotz	Senior Investigator	ARB NIADDK
	J.H. Klippel	Senior Investigator	ARB NIADDK
	J.E. Balow	Senior Investigator	ARB NIADDK
	Simon Carette	Investigator	ARB NIADDK

COOPERATING UNITS (if any)
None

LAB/BRANCH
Arthritis and Rheumatism Branch

SECTION
Connective Tissue Disease

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0.5	PROFESSIONAL: 0.5	OTHER: 0

CHECK APPROPRIATE BOX(ES)
☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Patients with diffuse glomerulonephritis and systemic lupus erythematosus satisfying criteria for active renal disease are offered a therapeutic study. The patients are randomized to different treatment programs which include the following drugs: corticosteroids, azathioprine and cyclophosphamide.

Follow-up visits occur every six months and the patients are followed until death or dialysis. Clinical and immunologic assessment are performed. The risks and benefits of each treatment will be evaluated and compared.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 41018-08 ARB

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Production of covalently cross-linked immune complexes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P.H. Plotz	Senior Investigator	ARB NIADDK
OTHER:	D. Finbloom	Clinical Associate	ARB NIADDK
	D. Magilavy	Clinical Associate	ARB NIADDK
	A. Rifai	Staff Fellow	ARB NIADDK

COOPERATING UNITS (if any)

None

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

Connective Tissue Disease

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 1.5	PROFESSIONAL: 1.5	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Three major aspects of immune complex biology have been been studied. First, the ability of the mouse liver to handle infused complexes was studied, using site-specifically cross-linked model complexes. The receptors for complexes on Kupffer cells were found to be specific for the immunoglobulin molecule and not other aggregated proteins removed by the same cells. Furthermore, NZB/W mice, with murine lupus, show an impaired ability to injest complexes after they are bound to Kupffer cells. Second, the influence of antigen or the handling of complexes has been studied using pairs of glycoprotein antigens differing only in their carbohydrate side chains but reacting with the same antibodies to form complexes. Desialylated glycoproteins carry complexes to hepatocytes, whereas complexes with the intact protein go to Kupffer cells. Third, the influence of monosaccharides substituted onto the Ficoll backbone of another group site-specifically cross-linking antigens has been studied. Galactose substitution on the antigen carries the stable complexes to hepatocytes, whereas mannose brings them to Kupffer cells.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AM 41020-15 ARB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Pathogenesis of autoimmunity in mice with SLE-like illness

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A.D. Steinberg	Senior Investigator	ARB NIADDK
OTHER:	T. Santoro	Medical Staff Fellow	ARB NIADDK
	P. Smathers	Biologist	ARB NIADDK
	J. Reeves	Chemist	ARB NIADDK
	E. Raveche	Chemist	LEP NIADDK
	J. Cowdery	Clinical Associate	ARB NIADDK
	C. Hansen	Geneticist	R VR
	C. Laskin	Visiting Fellow	ARB NIADDK
	H. Smith	Clinical Associate	ARB NIADDK
	M. Miller	Medical Staff Fellow	ARB NIADDK
	B. Steinberg	Student Volunteer	ARB NIADDK

COOPERATING UNITS (if any)
Laboratory of Experimental Pathology, NIADDK
Small Animal Section, VR

LAB/BRANCH
Arthritis and Rheumatism Branch

SECTION
Section on Cellular Immunology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5	4	1

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Autoimmunity in New Zealand mice has a genetic and immune basis; however, expression of severity can be modified by viral and other environmental factors as well as sex hormones. The immunologic factors are complex. These include excessive B-cell activation and abnormal T cell function. The xid gene codes for the deletion of a B cell subset. This gene prevents disease in congenic NZB and (NZB x NZW)F mice. BXSB mice have a Y chromosome-linked gene which accelerates autoimmunity in autoimmune mice, but does not cause it in non-autoimmune mice. All of the autoimmune strains studied have a defect in experimental tolerance. In NZB mice, the defect maps to a stem cell pre-T cell.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 41021-14 ARB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Antinuclear antibodies in spontaneous and drug-induced SLE and other diseases

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A.D. Steinberg	Senior Investigator	ARB NIADDK
OTHER:	J.S. Cowdery	Clinical Associate	ARB NIADDK
	E.S. Raveche	Chemist	LEP NIADDK
	J.P. Reeves	Chemist	ARB NIADDK
	P.A. Smathers	Biologist	ARB NIADDK
	H.R. Smith	Clinical Associate	ARB NIADDK
	M.L. Miller	Clinical Associate	ARB NIADDK
	B. Littman	Guest Worker	MB NCI
	B.J. Steinberg	Student Volunteer	ARB NIADDK

COOPERATING UNITS (if any)
Laboratory of Experimental Pathology, NIADDK

LAB/BRANCH
Arthritis and Rheumatism Branch

SECTION
Section on Cellular Immunology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 3.0	PROFESSIONAL: 2.5	OTHER: .5

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Antibodies to nucleic acids are important diagnostically and pathogenetically in systemic lupus. The production of these antibodies is in part controlled by the X-chromosome through regulation of maturation of B cells destined to make IgM antibodies to nucleic acids. Sex chromosomes indirectly influence the magnitude of auto-antibody production through sex hormones: male sex hormones suppress the production of anti-nucleic acid antibodies.

Hybridoma cells producing anti-nucleic antibodies [derived originally by fusing BALB/c myeloma line and (NZB x NZW) F1 spleen cells] preferentially grow and preferentially produce different autoantibodies in different recipient strains. These studies suggest that control mechanisms exist for the proliferation of, and perhaps synthesis by, B cells genetically destined to produce antibodies to nucleic acids. Antibodies specific for anti-RNA or anti-DNA antibodies can be produced and used to study the regulation of antibody production.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 41022-11 ARB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Therapeutic Studies in New Zealand Mice

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A.D. Steinberg	Senior Investigator	ARB NIADDK
OTHER:	J.P. Reeves	Chemist	ARB NIADDK
	P.A. Smathers	Biologist	ARB NIADDK
	H.R. Smith	Clinical Associate	ARB NIADDK
	R.T. Steinberg	Student Volunteer	ARB NIADDK
	D. Robinson		Harvard Medical School
	J. Prickett		Harvard Medical School

COOPERATING UNITS (if any)

Harvard Medical School

LAB/BRANCH
Arthritis and Rheumatism Branch

SECTION
Section on Cellular Immunology

INSTITUTE AND LOCATION
NIH, NIADDK, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Treatment of NZB/NZW mice with Ribavirin, a broad spectrum antiviral drug, has been very effective. Renal deposits of immune complexes can be prevented. Such treatment is potentially applicable to humans with SLE.

A diet enriched in a polyunsaturated fatty acid eicosapentaenoic markedly retards the development of autoimmunity in (NZB x NZW) F1 females. This works even after the onset of disease. Thymectomy markedly retards autoimmunity in MRL-lpr/lpr mice.

Cyclophosphamide is effective treatment in a variety of autoimmune strains unrelated to the cellular basis of illness or the nature (B or T cell) of the proliferating cells.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 41023-08 ARB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Study of Immune Abnormalities in Patients with Systemic Lupus Erythematosus

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A.D. Steinberg	Senior Investigator	ARB NIADDK
OTHER:	J.P. Reeves	Chemist	ARB NIADDK
	I. Green	Senior Investigator	LCI NIADDK
	R.M. Blaese	Senior Investigator	IB NIC
	S. Kumagai	Visiting Fellow	LCI NIAID
	J. Smolen	Visiting Fellow	ARB NIADDK
	C. Morimoto	Harvard Medical School, Boston	
	T. Chused	Senior Investigator	LMI NIAID
	K. Siminovitch	Visiting Fellow	ARB NIADDK
	T. Santoro	Medical Staff Fellow	ARB NIADDK

COOPERATING UNITS (if any)
Immunology Branch, NCI
Laboratory of Clinical Investigations, NIAID

LAB/BRANCH
Arthritis and Rheumatism Branch

SECTION
Section on Cellular Immunology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 2.0	PROFESSIONAL: 1.5	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Excessive B cell activity and effects in regulatory T cell function occur in patients with active SLE; these abnormalities are less marked when the disease is quiescent. Nevertheless, excessive B cell proliferation occurs even in inactive patients. Studies with monoclonal anti-T cell antibodies indicate that many patients have abnormal presence of activated T cells and increased numbers of B cells. Study of a large number of patients with SLE indicate that SLE is not a disease, but a syndrome with different cellular bases.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 41025-11 ARB

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Studies on the cell surface receptor for IgE

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. Metzger	Chief, Sec. Chem. Immunol.	ARB NIADDK
OTHER:	C. Fewtrell	Visiting Scientist	ARB NIADDK
	N. Kumar	Visiting Fellow	ARB NIADDK
	B. Rivnay	Visiting Fellow	ARB NIADDK
	R. Perez	Guest Worker	ARB NIADDK
	S. Wank	Research Associate	ARB NIADDK
	D. Finbloom	Clinical Associate	ARB NIADDK
	G. Rossi	Visiting Scientist	ARB NIADDK
	C. DeLisi	Senior Investigator	LTB NCI

COOPERATING UNITS (if any)

LTB/NCI

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

Chemical Immunology

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 8.0	PROFESSIONAL: 7.0	OTHER: 1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We are pursuing an in-depth investigation on the structure and function of the membrane receptor for IgE. For these studies intact cells from a rat basophilic leukemia as well as normal rat mast cells are being used. In addition receptor-bearing membrane vesicles and solubilized receptors are being studied. The aim is to understand the structure of the receptor as well as its integration in the plasma membrane. Results of such studies as well as direct functional studies are aimed at understanding the early stimulation of cells by antigen-induced, antibody-mediated reactions.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 41029-06 ARB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Development of Computerized Clinical Database for Systemic Lupus Erythematosus (SLE)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J.H. Klippel	Senior Investigator	ARB NIADDK
OTHER:	J.L. Decker	Chief	ARB NIADDK
	E.K. Harris	Chief	LAS DCRT
	G. Shakarji		DMB DCRT

COOPERATING UNITS (if any)
Laboratory of Applied Studies, DCRT
Data Management Branch, DCRT
Laboratory of Statistical and Mathematical Methodology, DCRT

LAB/BRANCH
Arthritis and Rheumatism Branch

SECTION
Connective Tissue Disease

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER
☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

PROJECT TERMINATED

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 41038-05 ARB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Renal biopsy pathology in systemic lupus erythematosus

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: J.E. Balow	Senior Investigator	ARB NIADDK

COOPERATING UNITS (if any)
Armed Forces Institute of Pathology, Washington, D.C.
(T.A. Antonovych, M.D.)

LAB/BRANCH
Arthritis and Rheumatism Branch

SECTION
Connective Tissue Disease

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Detailed analysis of renal biopsy pathology has been undertaken in patients with systemic lupus erythematosus who are receiving various therapeutic regimens of immunosuppressive agents. Biopsies will be scored for various indices of pathological activity as well as for patterns of deposition of immune complexes by immunofluorescence and electron microscopy; these morphologic parameters are to be related to renal function data in order to ascertain whether more accurate prognostic indices can be developed and whether more sensitive measures of therapeutic response can be identified.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 41040-10 ARB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Study of various cytotoxic drug programs in diffuse lupus nephritis (PACIV)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A.D. Steinberg	Senior Investigator	ARB NIADDK
OTHER:	J.L. Decker	Chief, Arthritis and Rheumatism Branch	ARB NIADDK
	P.H. Plotz	Senior Investigator	ARB NIADDK
	J.H. Klippel	Senior Investigator	ARB NIADDK
	J.E. Balow	Senior Investigator	ARB NIADDK
	R. Wilder	Senior Investigator	ARB NIADDK

COOPERATING UNITS (if any)

None

LAB/BRANCH
Arthritis and Rheumatism Branch

SECTION
Section on Cellular Immunology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Patients with the diffuse forms of systemic lupus erythematosus nephritis were invited to enter a therapeutic trial. The three programs were corticosteroids only; corticosteroids with cyclophosphamide and azathioprine, both in low doses; and corticosteroids with intermittent intravenous cyclophosphamide. Regular follow-up of the patients admitted continues. Long-term studies of patients in our earlier trials suggest that the degree of chronicity on the renal biopsy may provide important prognostic information. The subset of patients with moderate chronicity responds to oral azathioprine or oral cyclophosphamide when added to low doses of corticosteroids.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 41048-03 ARB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Study of Genetic and Pathophysiologic Features in the Rat of Experimental Erosive Synovitis Induced by Bacterial Cell Walls

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I:	R.L. Wilder	Senior Investigator	ARB NIADDK
OTHER:	C.T. Hansen	Chief	VR RR
	L.M. Wahl	Senior Investigator	LMI NIDR
	S.M. Wahl	Senior Investigator	LMI NIDR
	J.B. Allen	Biochemist	ARB NIADDK
	G.B. Calandra	Medical Director	Merck Sharp Dome Laboratories
	D. Blatter	Summer Student	ARB NIADDK

COOPERATING UNITS (if any)

Laboratory of Microbial Immunology, NIDR

LAB/BRANCH
Arthritis and Rheumatism Branch

SECTION
Connective Tissue Diseases

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 3	PROFESSIONAL: 2	OTHER: 1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Chronic Erosive Synovitis can be induced experimentally in rats by systemic administrations of cell walls from certain bacteria, such as group A streptococci. Genetic and hormonal factors determine susceptibility to arthritis. Susceptible inbred strains develop intense mononuclear cell inflammation in peripheral joints, liver and spleen. Resistant inbred strains develop mononuclear cell inflammation in the spleen but not the joints. The cell walls are poorly degraded and can be demonstrated in macrophages at the sites of inflammation for more than 16 weeks. Thus, susceptibility appears to reflect a host defect in mechanisms which limit translocation of the phlogistic cell walls to synovial tissue. The detailed mechanisms underlying this defect are under continuing investigation.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 41049-03 ARB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

The comparative efficacy and safety of azathioprine and D-penicillamine for the treatment of rheumatoid arthritis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. H. Plotz	Senior Investigator	ARB NIADDK
OTHER:	J. L. Decker	Chief, Arthritis & Rheum. Br.	ARB NIADDK
	J. H. Klippel	Senior Investigator	ARB NIADDK
	R. Wilder	Clinical Associate	ARB NIADDK

COOPERATING UNITS (if any)

None

LAB/BRANCH
Arthritis and Rheumatism Branch

SECTION
Connective Tissue Disease

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0.25	PROFESSIONAL: 0.25	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In this study, patients with rheumatoid arthritis unresponsive or intolerant to gold therapy will be randomized in a double-blind trial to receive either azathioprine or d-penicillamine. The trial will compare the efficacy and toxicity of these during a 24 week trial and an extended follow-up observation period. The Arthritis and Rheumatism Branch is one of eleven clinics carrying out this protocol under the direction of the Center for Cooperative Studies in the Rheumatic Diseases.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 41050-03 ARB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Comparative efficacy and safety of myochrysine and auranofin in the treatment of rheumatoid arthritis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P.H. Plotz	Senior Investigator	ARB NIADDK
OTHER:	J.L. Decker	Chief, Arthritis & Rheum. Br.	ARB NIADDK
	J.H. Klippel	Senior Investigator	ARB NIADDK
	R. Wilder	Clinical Associate	ARB NIADDK

COOPERATING UNITS (if any)
None

LAB/BRANCH
Arthritis and Rheumatism Branch

SECTION
Connective Tissue Disease

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0.25	PROFESSIONAL: 0.25	OTHER: 0

CHECK APPROPRIATE BOX(ES)
☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Gold has been known to have a beneficial effect on rheumatoid arthritis for many decades. In this study, a new oral gold preparation (auranofin) will be compared to a placebo and to intramuscular gold in a double-blind trial involving 11 clinics cooperating under the aegis of the Center for Cooperating Studies in the Rheumatic Diseases.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 41051-03 ARB

PERIOD COVERED
October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)

Natural History and in situ organization of the receptors for IgE.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. Isersky-Carter	Senior investigator	ARB NIADDK
Others:	H. Metzger	Chief, Sec. on Chem. Immunol.	ARB NIADDK

COOPERATING UNITS (if any)

R. Siraganian LMI, NIDR., L. Basciano, T. Triche L.P., NCI

LAB/BRANCH
Arthritis and Rheumatism Branch

SECTION
Chemical Immunology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We are investigating the natural history and in situ organization of the receptor for IgE on mast cells and basophils. Various sublines of rat basophilic leukemia (RBL) cells and plasma membrane vesicles prepared from them are used. Purified IgE myeloma, antibodies to IgE and to the receptor are also used. These studies will help clarify the mode of function of the receptor, the fate of the receptor-bound monomeric and cross-linked IgE and the turnover of the free receptor.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01AM41052-03 ARB

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Disorders of immune regulation in patients with systemic lupus erythematosus

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. E. Balow	Senior Investigator	ARB NIADDK
Other:	G. C. Tsokos	Guest Worker	ARB NIADDK
	C. B. Christian	Biologist	CC

COOPERATING UNITS (if any)

None

LAB/BRANCH
Arthritis and Rheumatism Branch

SECTION
Connective Tissue Disease

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 2.0	PROFESSIONAL: 1.0	OTHER: 1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Patients with systemic lupus erythematosus have been found to have disturbances of cell-mediated immune responses. Alterations of B and T lymphocytes as well as monocyte function, natural killer cell and interferon responses, have been observed both in patients with SLE and certain of their relatives. The major goal of these studies is to further characterize the types of immunological disturbances in SLE and to elucidate the mechanisms which lead to alterations of immune regulation and are responsible for the pathogenesis of this disease. The ability of immunosuppressive drugs to alter cell mediated immune function and possibly to re-establish more nearly normal immunoregulation is being actively studied.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 41053-03 ARB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Effects of High-Dose Intravenous Methylprednisolone on the Course of Rheumatoid Arthritis (RA)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R.L. Wilder	Senior Investigator	ARB NIADDK
Other:	J.H. Klippel	Senior Investigator	ARB NIADDK
	J.L. Decker	Chief	ARB NIADDK
	A.S. Fauci	Senior Investigator	LCI NIAID
	P.H. Plotz	Senior Investigator	ARB NIADDK
	N.L. Gerber	Chief	REHAB CC
	P.K. Navang	Senior Staff Fellow	CPRL PDS CC

COOPERATING UNITS (if any)

None

LAB/BRANCH
Arthritis and Rheumatism Branch

SECTION
Connective Tissue Diseases

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 2.0	PROFESSIONAL: 2.0	OTHER: 2.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

PART ONE-Six patients with rheumatoid arthritis (RA) unresponsive to conventional therapies have been studied in an open pilot trial of short-term pulse methylprednisolone (MP). Following administration of 1 gram of MP intravenous on 3 consecutive days, dramatic changes in articular index were evident in the first 1-2 weeks. The effect persisted for a variable period of time - 2 weeks to more than 8 weeks. No major side effects were observed. No persistent changes in several immunological parameters were measured. The results provide a preliminary basis to design better therapeutic protocols.

PART TWO-Oral administration of high dose pulse corticosteroid is potentially a more practical method of administering pulse therapy. Drug absorption and patient acceptance, however, of high dose orally administered steroids are not well documented. Thus, we studied plasma MP levels in 3 patients with RA after a single IV or oral administration. Results indicate that orally administered high dose MP is well absorbed (> 60%.) and well tolerated, but therapeutic equivalence of IV versus orally administered MP is not certain.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 41054-02 ARB

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

The Effect of a Brief Course of Leukapheresis in the Management and Investigation of Rheumatoid Arthritis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R.L. Wilder	Senior Investigator	ARB NIADDK
OTHER:	J.L. Decker	Chief	ARB NIADDK
	L. Wahl	Senior Investigator	LMI NIDR
	S. Wahl	Senior Investigator	LMI NIDR
	C. Yarboro	Clinical Nurse	ARB NIADDK
	I. Scher	Senior Investigator	NMRI NNMC
	I. Catona	Senior Investigator	NMRI NNMC
	J.B. Allen	Biochemist	ARB NIADDK

COOPERATING UNITS (if any)

None

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

Connective Tissue Disease

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.0	3.5	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The effects of a brief course of leukapheresis were studied in eleven patients with Rheumatoid Arthritis, unresponsive to conventional therapy. The patients consisted of two groups--those with profoundly depressed or normal proliferation to soluble antigens. They represented the extremes of our rheumatoid patient population. Six of 7 patients with depressed proliferation clinically improved, whereas 0/4 patients with normal proliferation clinically improved. This and other immunologic abnormalities tended to normalize in the group which clinically improved. Thus, reversal of depressed immune function appeared to parallel clinical improvement. These studies provide new insights into the pathogenesis of rheumatoid arthritis and more insight into the effects of leukapheresis.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 41055-02 ARB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
The Effect of Plasmapheresis on Systemic Lupus Erythematosus (SLE)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I:	J.H. Klippel	Senior Investigator	ARB NIADDK
OTHER:	J.E. Balow	Senior Investigator	ARB NIADDK
	T.H. Lawley	Senior Investigator	DB NCI
	R.P. Hall	Senior Investigator	DB NCI
	P.H. Plotz	Senior Investigator	ARB NIADDK
	A.D. Steinberg	Senior Investigator	ARB NIADDK
	Emilie Klima	Technician	ARB NIADDK
	Cheryl Yarboro	Nurse	CC
	W. Campbell	Nurse	CC
	J.L. Decker	Chief	ARB NIADDK

COOPERATING UNITS (if any)
Dermatology Branch, NCI

LAB/BRANCH
Arthritis and Rheumatism Branch

SECTION
Connective Tissue Diseases

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0.25	PROFESSIONAL: 0.25	OTHER: 0

CHECK APPROPRIATE BOX(ES)
☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER
☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The therapeutic efficacy of short-term plasmapheresis in SLE will be assessed in a double-blind randomized trial. Patients will receive either 4 liter plasmapheresis or reinfusion plasmapheresis 3 times weekly for a period of 2 weeks. Clinical and laboratory assessments of lupus activity will be done with each apheresis and every week subsequent for 4 weeks. The protocol will be completed at the end of the 6th week or if the patient is withdrawn as a clinical therapeutic failure.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 41056 - 02 ARB

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Immunosuppressive Drug Therapy in Lupus Glomerulonephritis (LUPULSE)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	J. E. Balow	Senior Investigator	ARB NIADDK
Other	J. L. Decker	Chief, ARB	ARB NIADDK
	J. H. Klippel	Senior Investigator	ARB NIADDK
	P. A. Plotz	Senior Investigator	ARB NIADDK
	A. D. Steinberg	Senior Investigator	ARB NIADDK
	R. L. Wilder	Senior Investigator	ARB NIADDK

COOPERATING UNITS (if any)

None

LAB/BRANCH
Arthritis and Rheumatism Branch

SECTION
Connective Tissue Disease

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 1.50	PROFESSIONAL: 1.25	OTHER: 0.25

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The efficacy of intensive, intermittent immunosuppressive drug therapy will be evaluated in patients with active lupus glomerulonephritis over a 30 month study period. Patients with renal biopsy documented active glomerulonephritis with or without renal functional deterioration will be treated with low dose corticosteroids and randomized to receive (a) intravenous methylprednisolone (1.0 gm/m^2 monthly for 6 months or (b) intravenous cyclophosphamide 1.0 gm/m^2 monthly for 6 months or (c) intravenous cyclophosphamide 1.0 gm/m^2 monthly for 6 months and then every 3 months for the remaining 24 months of the study. During the final 24 months of the study, all patients will receive low dose prednisone. Active disease, as manifested by renal functional deterioration, increased proteinuria or worsened urinary sediment, will be treated by increased prednisone. Comparison will be made of the number of favorable outcomes of renal functions and glomerular pathology and drug related toxicities achieve by each treatment group at the end of the 6th and 30th study months.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 41057-01 ARB

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Low Dose Methotrexate Therapy in Rheumatoid Arthritis (RA)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: J.H. Klippel, M.D., Senior Investigator, ARB:NIADDK

Other:

John L. Decker	Chief	ARB NIADDK
Paul H. Plotz	Senior Investigator	ARB NIADDK
Ronald L. Wilder	Senior Investigator	ARB NIADDK
Lynn H. Gerber	Chief	REHAB CC
Cheryl Yarboro	Nurse	CC

COOPERATING UNITS (if any)

Rehab, CC

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

Connective Tissue Diseases

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0.25	PROFESSIONAL: 0.25	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study will evaluate the benefits and incidence and severity of side effects of low dose pulse oral methotrexate in patients with rheumatoid arthritis in a double-blind study. Fifteen patients will be randomized to receive either low dose oral methotrexate (7.5-15mg/week) or placebo for 18 weeks. The Arthritis and Rheumatism Branch is one of eleven clinics carrying out this protocol under the direction of the Cooperating Clinics for the Systematic Studies of Rheumatic Diseases.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 41058-01 ARB

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Pulse Electromagnetic Field Therapy of Osteonecrosis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I:	J.H. Klippel	Senior Investigator	ARB NIADDK
	L.H. Gerber	Chief	REHAB CC
OTHER:	John L. Decker	Chief	ARB NIADDK
	Anne Brower	Assoc. Prof.	USUHS
	Darrell W. McIndoe	Asst. Prof.	USUHS
	H.S. Cattell	Surgeon	NIH CONSULTING STAFF

COOPERATING UNITS (if any)

Rehab, CC

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

Connective Tissue Disease

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The effects of pulse electromagnetic field (PEMF) therapy in osteonecrosis will be evaluated in a randomized, double-blind study. Twenty patients with symptomatic osteonecrosis of either (or both) the femoral head or femoral condyle will be studied. Patients will be provided with molded casts containing electromagnetic coils and randomized to receive either PEMF (treatment group) or no PEMF (control group) across the site of involvement. The external casts with or without PEMF will be applied to the involved area for 10 hours daily for a total of six months. Patients will be evaluated at 3 and 6 months by questionnaire, joint examination, bone scintigraphy and X-rays for evidence of healing of the osteonecrosis.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 41059 - 01 ARB

PERIOD COVERED
October 1, 1981 through September 30 1982

TITLE OF PROJECT (80 characters or less)
Immunosuppression and Plasmapheresis in Goodpasture's Syndrome

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.	J. E. Balow	Senior Investigator	ARB NIADDK
	H. A. Austin	Expert.	C.C.

COOPERATING UNITS (if any)
Dr. John Johnson, Walter Reed Army Medical Center
Dr. Curtis Wilson, Scripps Clinic and Research Foundation

LAB/BRANCH
Arthritis and Rheumatism

SECTION
Connective Tissue Disease

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0.25	PROFESSIONAL: 0.25	OTHER: 0

CHECK APPROPRIATE BOX(ES)
☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER
☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Goodpasture's Syndrome is usually characterized by rapidly progressive glomerulonephritis due to autoantibodies against glomerular basement membrane (anti-GBM). This study will examine whether plasmapheresis significantly improves the results of standard immunosuppressive drug treatment of this disease. Patients will be randomly allocated to drug treatment alone or drugs plus plasmapheresis until anti-GBM levels are undetectable. Comparison will be made of the numbers of favorable outcomes and complication rates of these therapies at the end of 6 months of study.

ANNUAL REPORT OF THE METABOLIC DISEASES BRANCH

The general goals of the Branch are to investigate the structure, secretion and biochemical mechanism of action of hormones governing ion transport and mineral metabolism and to apply the knowledge gained to understanding and treating related clinical disorders. Current investigations include hormone-receptor interaction, mechanism of receptor and guanine nucleotide control of adenylate cyclase, cyclic AMP control of ion transport, regulation of parathyroid hormone secretion, and receptor for biologically active vitamin D metabolites. Systems under study include β-adrenergic receptors in several tissue, isolated parathyroid cell systems and skeletal and renal tissue. Clinical studies on hyperparathyroidism and hormone-resistant states continue to utilize extensively the research findings from the laboratory. 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) receptor defects have been identified in skin fibroblasts from cases of osteomalacia resistant to $1,25(OH)_2D$. A deficiency of the guanine nucleotide regulatory protein has been discovered in pseudohypoparathyroidism. Radioimmunoassays for parathyroid hormone have been applied to determine the hormone in samples obtained selectively by venous catheterization, thereby allowing localization of parathyroid adenomas. Further studies make use of urinary cyclic AMP clearance as a clinical parameter of parathyroid hormone action. Another investigation takes advantage of the autologous parathyroid transplant program developed in collaboration with the Surgery Branch, NCI. Autologous transplants to the arm allow direct sampling of draining veins for parathyroid hormone secretion in man under conditions wherein the tissue can be selectively challenged with potential secretagogues or inhibitors of parathyroid secretion.

β-Adrenergic Receptor of the Avian Erythrocytes.

Interactions of catecholamines with β-adrenergic receptors and activation of adenylate cyclase are under study with the plasma membranes of turkey erythrocytes and other cell systems. Specific receptors have now been identified on turkey erythrocytes, parathyroid cells, pineal cells, rat, guinea pig and monkey lung membrane preparations and rat liver membranes.

β-Adrenergic receptor sites are identified using a radioiodinated β-blocker iodo-hydroxybenzylpindolol. A series of β-adrenergic agonists and inhibitors compete for binding with affinities paralleling biological effectiveness as activators or inhibitors of catecholamine-stimulated adenylate cyclase. Receptor occupancy for agonists correlates with function (cyclic AMP production). The stereospecificity of interaction with the iodinated β-blocking agent and the correspondence between affinity and biological effectiveness indicate that binding of iodohydroxybenzylpindolol validly reflects interaction with the β-adrenergic receptor.

Receptors for Calcitonin and Parathyroid Hormone

Studies in collaboration with Dr. T. Murray (St. Michael's Hospital, Toronto) have led to development of radiolabeled intact bovine parathyroid hormone as a ligand. This material binds to receptors in canine renal plasma membranes or in cultured osteosarcoma cells with kinetic properties distinct from those of radiolabeled synthetic amino terminal bioactive fragments. Thus the new ligand should prove useful for evaluating PTH-receptor interactions of the native hormone.
[Drs. Marx, Aurbach and Rizzoli]

Guanine Nucleotide Regulatory Protein

Studies on the role of guanine nucleotides in the control of adenylate cyclase activity are continuing. Guanine nucleotides enhance catecholamine-stimulated adenylate cyclase activity in turkey erythrocyte membranes. This action is mediated by a guanine nucleotide binding regulatory protein which is distinct from the receptor and the catalytic unit of adenylate cyclase. In the turkey erythrocyte membrane system, exchange of guanine nucleotides bound to the regulatory protein is strictly dependent on the action of hormone (β-adrenergic agonists). However, treatment of membranes with isoproterenol and GMP (a nucleotide bound with low affinity) permits later hormone-dependent activation and manipulation of the regulatory protein. After isoproterenol and GMP treatment, the guanine nucleotide regulatory protein is extracted from the membrane by Lubrol PX, and adsorbs to a GTP affinity matrix, from which it is eluted by high concentrations of guanine nucleotides. The regulatory protein, purified 50-100 fold by affinity adsorption, restores both guanine nucleotide- and fluoride-responsive adenylate cyclase activity to membranes from a mutant (CYC$^-$) strain of S49 mouse lymphoma which lacks the regulatory protein.

The guanine nucleotide regulatory protein from turkey erythrocytes is covalently radiolabeled by the cholera toxin-catalyzed transfer of [^{32}P]ADP-ribose from [^{32}P]NAD to a 42,000 M_r subunit of the regulatory protein. [^{32}P]ADP-ribosylation permits detection of the regulatory protein during detergent extraction, affinity purification, sucrose gradient centrifugation, and manipulations which cause inactivation of the regulatory protein. Radiolabeling of the regulatory protein should permit more accurate quantitation of enrichment during purification. Similar techniques applied to human tissue may permit detection of quantitative and qualitative alteration in hormone-resistant states such as pseudohypoparathyroidism.

Changes in the nature of the guanine nucleotide bound to the regulatory unit influence fluoride-stimulated adenylate cyclase activity. Incubation of turkey erythrocyte membranes with isoproterenol and GMP clears many of the regulatory sites of tightly bound GDP, substituting GMP. Fluoride-stimulated adenylate cyclase activity declines during isoproterenol and GMP treatment, but is restored by addition of GTP or GDP. Studies performed with the GDP analogs GP(CH)$_2$P and GDP-β-S, and direct analysis of the nucleotides bound at isoproterenol-dependent regulatory sites during fluoride activation, indicate that fluoride does not enhance conversion of GDP to GTP at regulatory sites, nor does it inhibit hydrolysis of GTP to GDP. Also, exchange of guanine nucleotide bound to the regulatory protein with nucleotides in the medium does not occur during enzyme activtion by fluoride.

Studies with partially purified, affinity eluted, guanine nucleotide regulatory protein confirm that fluoride stimulation of adenylate cyclase depends on the guanine nucleotide bound to the regulatory unit. Regulatory protein eluted with either GTP or GDP restores fluoride-stimulated enzyme activity to the S49 CYC$^-$ acceptor, but regulatory protein eluted with GMP restores fluoride-stimulated activity only when GTP or GDP were added, indicating that the guanine nucleotide regulatory protein, and an appropriate nucleotide, are necessary for fluoride-stimulated adenylate cyclase activity.

Cholera toxin catalyzes [^{32}P]ADP-ribosylation of the 42K subunit of the G unit. Limited proteolysis generates fragments detected by autoradiography

after SDS-gel electrophoresis. Unique proteolytic fragments are generating depending upon whether an active (e.g. GTP-γ-S) or inactive (e.g. GDP) guanine nucleotide is bound to the G unit. Identical results are obtained with membranes possessing the catalytic unit and with affinity eluate, lacking catalytic unit. These results point to a guanine nucleotide-directed change in conformation of the G unit and correspond to changes in apparent size of the G unit (as assessed by sucrose density gradient centrifugation) induced by GDP vs GTP-γ-S.
[Drs. Spiegel, Downs, Levine and Aurbach, NIADDKD]

Vanadate, an anion with important effects on Na, K-ATPase, also stimulates adenylate cyclase. We have shown that as with fluoride, stimulation by vanadate requires the G unit. Vanadate and fluoride stimulation differ, however, in their susceptibility to inhibition by cholera toxin and the GDP analog GDP-β-S.
[Drs. Krawietz, Spiegel, Downs and Aurbach]

The mechanism of calcium inhibition of adenylate cyclase was studied using partially separated components of the enzyme complex and analysis of free calcium concentration. The K_i for inhibition of the enzyme by free calcium was 2.48 x 10^{-4}M. Calcium did not affect hormone-stimulated guanine nucleotide exchange, conformation of the activated regulatory (G unit) protein or of the catalytic unit. Neither did calcium affect dissociation of activated G unit from catalytic unit nor convert activated catalytic unit to an inactive form. Evidence was found that calcium inhibits the catalytic subunit of adenylate cyclase by direct competition with magnesium for its regulatory site.
[Drs. Lasker and Aurbach]

Pseudohypoparathyroidism

The molecular defect in pseudohypoparathyroidism (PHP) has been investigated using techniques adapted from basic studies on the turkey erythrocyte adenylate cyclase complex. Patients with PHP can be divided into two phenotypes, i.e. those showing features of Albright's hereditary osteodystrophy (+AHO) and those lacking such features (-AHO). We have assayed the guanine nucleotide regulatory unit (G unit) in red cell membranes from about 30 patients with PHP and found that +AHO patients generally show a 50% reduction in functional G unit while -AHO patients show a normal G unit complement. G unit deficiency (demonstrated by assay with exogenous adenylate cyclase catalytic units and by cholera toxin-dependent ADP-ribosylation) is also evident in cultured fibroblast and renal cortical plasma membranes from patients with PHP + AHO. These findings support the hypothesis that a generalized deficiency of G unit activity is the cause of hormone resistance in some patients with PHP. Also consistent with this hypothesis is the finding that adenylate cyclase activity in cultured fibroblast plasma membranes and cAMP accumulation in intact fibroblasts are lower in patients with PHP than in normal controls. *In vivo* studies of responsiveness to TSH and glucagon show that patients with PHP and G unit deficiency are resistant to multiple hormones while patients with PHP and normal G unit activity show resistance limited to PTH.
[Drs. Spiegel, Levine, Downs, Marx and Aurbach]

Primary Hyperparathyroidism and Familial Hypercalcemia

Clinical studies are continuing on primary hyperparathyroidism and its familial variants. Detailed family screening and case finding has produced 52 kindreds for analysis. These studies have allowed segregation of the familial variants into two apparently distinct disease syndromes -familial multiple endocrine adenomatosis (MEN I) and familial hypocalciuric hypercalcemia (FHH).

FHH was distinguished from MEN I by 1) virtually a 100% penetrance for hypercalcemia before age 20, 2) milder clinical manifestations - low incidence of recurrent nephrolithiasis or recurrent peptic ulceration, 3) no hypercalciuria, 4) normal basal concentrations of gastrin, and 5) poor response to subtotal parathyroidectomy. Distinction between the two syndromes, both inherited as autosomal dominant traits, is important because in FHH the clinical course is generally milder and subtotal parathyroidectomy is less likely to be beneficial. FHH accounts for approximately 10% of all unsuccessful parathyroidectomies in hypercalcemia. In FHH the ionized and ultrafiltrable calcium concentration in serum is elevated in proportion to the increase in total calcium. In these patients the filtrable load of calcium is high in association with a marked decrease in renal calcium clearance. The concentration of parathyroid hormone in plasma is lower in patients with FHH than in typical primary hyperparathyroid patients with similar degrees of hypercalcemia whether assessed by PTH radioimmunoassay or by renal clearance of cAMP or phosphate. The parathyroid glands show hyperplasia in most cases. In several kindreds one or more members have exhibited life-threatening primary hyperparathyroidism in the neonatal period. This may result sometimes from a double dose of the FHH gene. The plasma and urine cAMP response to PTH infusion is greater in FHH than in typical primary hyperparathyroidism. Even when these patients become surgically hypoparathyroid, the low renal clearance of calcium is strikingly persistent during calcium infusion. [Drs. Marx, Spiegel, Downs, Levine, Lasker, Santora and Aurbach]

Studies on modes of localizing parathyroid tumors continue. A high degree of success has been obtained in localizing tumors through vascular catheterization procedures. Parathyroid arteriography developed and performed by Dr. John Doppman afforded, in approximately 45% of cases tested, the identification of abnormal masses of tissue subsequently identified as parathyroid. Confirmation of the mass thus identified as parathyroid tissue can be accomplished by verifying high concentrations of parathyroid hormone by radioimmunoassay in veins draining the lesion. Draining vessels contain concentrations of hormone 2- to 20-fold or more greater than the concentration of parathyroid hormone detected by immunoassay in the peripheral plasma. In cases with single adenomas, this concentration ratio is greatest selectively in the one vein draining the lesion. This technique utilizing specific radioimmunoassays and selective catheterization is applicable also to determining the source of hormone in ectopic hyperparathyroidism produced by non-parathyroid cancers. Localization of anterior mediastinal parathyroid adenomas has become possible using computed tomography. Eight mediastinal adenomas have been treated nonsurgically by percutaneous injection via catheter occlusive agents into the arterial blood supply with 7 complete and one partial remissions.

A new series of radioligands for parathyroid hormone radioimmunoassay has been developed, using synthetic sequences and analogs from the midregion of human PTH. These reagents have led to development of sensitive and practical radioimmunoassays. Using these assays and gel filtration of plasmas, we have demonstrated that certain hyperfunctioning parathyroid glands release a previously unrecognized large PTH fragment containing the midregion but deficient in carboxy-terminal immunoreactivity.

We have studied a patient with parathyroid carcinoma who showed markedly elevated serum concentrations of the glycoprotein hormone subunits hCG-α and hCG-β, which fell dramatically after surgical removal of malignant tissue.

Screening of a large number of patients with different forms of hyperparathyroidism has shown these subunits to be a marker for malignancy in parathyroid disease or for islet cell involvement in familial multiple endocrine adenomatosis (MEN I).
[Drs. Marx, Spiegel, Downs and Aurbach, MDB, NIADDKD; Drs. Weintraub and Rosen, CEB, NIADDKD]

The role of $1,25(OH)_2D_3$, the most potent natural metabolite of vitamin D, has been assessed in hypocalcemic states. This very rapidly acting drug has simplified the management of hypocalcemia following parathyroidectomy; during this time skeletal remineralization imposes large but rapidly diminishing requirements for calcium.

We have evaluated patients with extreme resistance to $1,25(OH)_2D$. This can be a transient state as following parathyroidectomy or a permanent state as in familial cases. We have evaluated ten patients with familial resistance to $1,25(OH)_2D$. Most patients have hypocalcemic rickets, usually with associated total alopecia. Mineral homeostasis is usually improved by treatments that sustain $1,25(OH)_2D$ levels at 10-100 times normal.

Specific intracellular defects have been evaluated using cultured skin fibroblasts from these patients. With skin fibroblasts cultured from normals, a typical $1,25(OH)_2D$-receptor can be identified by binding in soluble extracts, by nuclear uptake of hormone with intact cells, or by density gradient analysis of labeled hormone in extracts of cytosol or nucleus. Fibroblasts from patients with familial resistance to $1,25(OH)_2D$ have shown a spectrum of defects including nonfunctional receptors, diminished numbers of receptors, and apparently normal receptors. The association of alopecia with multiple distinct genetic mutations in the $1,25(OH)_2D$ effector system indicates that $1,25(OH)_2D$-resistance is one direct cause of total alopecia.
[Drs. Marx and Liberman, MDB, NIADDK; Drs. Eil and Gamblin, BNMC; Dr. Balsan, Paris; Dr. Rosen, Albert Einstein; Dr. Singer, USC]

Rapid determination of intraoperative U_{cAMP} excretion (using the Gammaflo machine for <7 min cAMP radioimmunoassay) has proven to be a valuable tool in guiding surgery for primary hyperparathyroidism. Persistent elevation of U_{cAMP} requires continued search for abnormal tissue even after 1 or more abnormal glands have been removed. A rapid (mean 1.5 hours) drop in U_{cAMP} to the normal range obviates the need for continued exploration even in cases where histologic confirmation of parathyroidectomy is lacking. Spurts in U_{cAMP} above baseline may provide a clue to the location of abnormal parathyroid tissue.
[Drs. Spiegel, Marx, Downs and Aurbach, NIADDKD: Drs. Saxe and Brennan, NCI Surgery]

Determination of urinary cAMP excretion postoperatively in patients undergoing neck exploration for primary hyperparathyroidism is a useful method for assessing postoperative parathyroid function. U_{cAMP} excretion declines postoperatively in all patients in whom hypercalcemia is corrected but not in those with persistent hypercalcemia. In patients becoming severely hypocalcemic (and requiring vitamin D therapy) postoperatively, U_{cAMP} measurement enables one to distinguish patients with decreased parathyroid reserve as the cause for hypocalcemia (low U_{cAMP} excretion) from patients with healing osteitis fibrosa ("hungry bones") as the basis for hypercalcemia. U_{cAMP} in the latter group is often elevated

but can be suppressed if serum calcium is normalized. Elevated U_{cAMP} excretion postoperatively in the face of hypocalcemia enables one to predict that vitamin D therapy will be required temporarily and precludes the need for parathyroid autografts.
[Drs. Spiegel, Marx, Downs, Levine, Lasker and Aurbach, NIADDKD]

Studies have continued with dispersed bovine and human parathyroid cells. PTH secretion from parathyroid glands in vivo and cells in vitro is inhibited by calcium. Calcium may also modulate synthesis and degradation of PTH. This control exerted by calcium may be changed in certain pathologic states (glandular adenomas, carcinomas and perhaps hyperplasia). Several earlier studies have strongly suggested that cAMP is an intracellular mediator of PTH secretion; agents which alter cellular cAMP change PTH secretion in the same direction. Calcium decreases cellular cAMP although some of its effects may be independent of cAMP. The adenylate cyclase of the parathyroid cell is a potential regulatory site for the action of calcium. Adenylate cyclase in homogenates of dispersed parathyroid cells shows sensitivities to Ca (1/2 maximal inhibition at approximately 0.2 mM added calcium) that are similar in human parathyroid adenomas, carcinomas or hyperplastic glands and like that of normal bovine glands. There was no correlation between the in vitro calcium suppressibility of PTH release by dispersed cells (several of the adenomas and the one carcinoma were not suppressed by high extracellular calcium) and the sensitivity of the adenylate cyclase in the broken cell preparation. This suggests that the adenylate cyclase responsiveness to calcium is not the site of derangement in these pathological states.
[Drs. Spiegel, Lasker and Aurbach]

Since cAMP, an important intracellular mediator of PTH secretion, functions through regulation of protein phosphorylation, we sought to identify endogenous substrates in bovine parathyroid cells for cAMP-dependent protein kinase. We found two low molecular weight proteins ($\sim M_r$ = 19K and 15K) whose phosphorylation is stimulated significantly by agonists that increase parathyroid cellular cAMP (e.g. β-adrenergic agents) as well as cAMP itself. The onset and reversibility of cAMP-stimulated phosphorylation of these proteins correlates well with effects on PTH release. These studies suggest that these proteins are linked to the release mechanism for PTH.
[Drs. Lasker and Spiegel]

Humoral Hypercalcemia of Malignancy

A Leydig cell transplantable tumor causes hypercalcemia in rats. cAMP is increased in the urine of the tumor-bearing animals but plasma PTH is undetectable. Thus hypercalcemia apparently can be attributed to a non-parathyroid tumor factor that may, like parathyroid hormone, act through the adenylate cyclase-cyclic AMP system. This humoral factor, whose identity we wish to establish, presumably causes hypercalcemia by resorption of bone. Tumor cells cultured in vitro elaborate into the medium, a bone resorbing substance. Histomorphometric studies of bone from tumor-bearing and control animals show that the tumor factor causes extensive osteoclastic bone resorption and results in a major loss in trabecular bone. In addition, tetracycline labeling studies demonstrate a marked decrease in bone formation in tumor-bearing rats.
[Drs. Spiegel, Santora, Saxe, Deftos, Heersche, Downs, Marx and Aurbach]

Elevated nephrogenous cAMP has been found in the urine of patients with the syndrome of humoral hypercalcemia of malignancy. The relationship between

the increased urine cAMP and the factor causing bone resorption and hypercalcemia in such patients is controversial. Some groups report elevated nephrogenous cAMP in normocalcemic cancer controls. We have initiated an extensive study of cancer patients to evaluate urinary cAMP as a specific marker for the syndrome of humoral hypercalcemia of malignancy. The results should bear on the pathogenesis of this syndrome and the specificity of urinary cAMP as a diagnostic test.
[Drs. Spiegel, Santora and Marx, NIADDK; Dr. R. Fisher, NCI]

Clinical Studies in Osteoporosis

The cooperative study in Jerusalem of 300 ambulatory patients with osteoporosis is nearly complete. Preliminary analysis of the trial of high calcium, high phosphate and sodium fluoride has revealed the following: a decrease in bone density was observed in 30 percent of the osteoporotic patients treated with placebo for 3 years; no decrease in bone density was observed in the osteoporotic patients receiving $CaHPO_4$ and NaF for 2 years, and in 19 percent of this group slight increase in bone density was seen.

The Physics Department of Hadassah Medical Organization has been involved in developing the relatively new technique of Compton Scattering for measurement of bone density and has made a comparative study of it with photon absorptiometry. For Compton Scattering, the coefficient of variation among individuals was less than for photon absorptiometry, and the Compton Scattering technique detected a significant difference in bone density between osteoporotic and non-osteoporotic patients.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 43001-17 MD

PERIOD COVERED
October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)
Studies in Bone Metabolism

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

G. D. Aurbach, M.D., Chief, Metabolic Diseases Branch, NIADDKD
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S. J. Marx, M.D., Senior Investigator, MDB, NIADDKD
A. M. Spiegel, M.D., Senior Investigator, MDB, NIADDKD
J. Shapiro, M.D., Acting Director, Clinical Center, NIH.

COOPERATING UNITS (if any)
Osteoporosis and Fracture Research Institute, Hadassah Medical Organization, Jerusalem, Israel

LAB/BRANCH
Metabolic Diseases Branch

SECTION

INSTITUTE AND LOCATION
NIADDKD, NIH, Bethesda, Md. 20205

TOTAL MANYEARS: .25	PROFESSIONAL: .25	OTHER:

CHECK APPROPRIATE BOX(ES)
☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objectives of the project on bone metabolism are to investigate the factors influencing mineral storage and loss in demineralizing bone diseases and to study the influence of added calcium and phosphate on resorption of bone mineral. The project involves estimation of bone density by photon absorptiometry and various techniques relative to vitamin D and fluoride metabolism.

During the past year, clinical studies of osteoporosis have been continued, using photon absorptiometry and Compton Scattering to determine the possible effect of dietary manipulation on bone density. Preliminary analysis of the data suggests a slight positive effect on bone density from di-calcium phosphate and sodium fluoride.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 43002-17 MD

PERIOD COVERED
October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)
Structure, Secretion and Mechanism of Action of Parathyroid Hormone

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT
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S. J. Marx, M.D., Senior Investigator, MDB, NIADDKD
A. M. Spiegel, M.D., Senior Investigator, MDB, NIADDKD
R. W. Downs, Jr., M.D., Senior Clinical Associate, MDB, NIADDKD
M. A. Levine, M.D., Medical Staff Fellow, Endocrinology & Genetics, MDB, NIADDK
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A. Santora, M.D., Ph.D., Medical Staff Fellow, MDB, NIADDK
Jeffrey Norton, M.D., Surgery Branch, National Cancer Institute
J. T. Potts, Jr., M.D., Massachusetts General Hospital, Boston
H. T. Keutmann, M.D. "
M. Rosenblatt, M.D. "
T. Murray, M.D., Visiting Scientist, MDB, NIADDK

COOPERATING UNITS (if any)
Endocrine Unit, Massachusetts General Hospital

LAB/BRANCH
Metabolic Diseases Branch

SECTION

INSTITUTE AND LOCATION
NIADDKD, NIH, Bethesda, Md. 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.50	1.50	1.00

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

There is still much to be learned about the nature of circulating parathyroid hormone in clinical disease. It is the purpose of this project to study the function and mechanism of action of parathyroid hormone, study its relationship to human disease, and develop clinically useful tests for circulating parathyroid hormone. From these studies it is expected that one can understand the pathophysiology of certain metabolic diseases of bone and endocrine disturbances. The entire structures of bovine, porcine and human parathyroid hormone have been determined. Synthetic polypeptides representing the first 34 residues of bovine and human parathyroid hormone have been prepared. These molecules show all the biological properties of the native hormonal polypeptide. Highly sensitive radioimmunoassays for the hormone have been developed and are being modified further for improved clinical diagnostic parameters. Studies show that the mechanism of action of the hormone is mediated through direct hormonal activation of adenylate cyclase in bone and kidney. An isolated parathyroid cell system has been developed that allows studies of secretagogues for parathyroid hormone.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 43003-17 MD

PERIOD COVERED
October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)
Studies on the Mode of Action of Thyrocalcitonin

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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S. J. Marx, M.D., Senior Investigator, MDB, NIADDKD

COOPERATING UNITS (if any)

None

LAB/BRANCH
Metabolic Diseases Branch

SECTION

INSTITUTE AND LOCATION
NIADDKD, NIH, Bethesda, Md. 20205

TOTAL MANYEARS: .50	PROFESSIONAL: .25	OTHER: .25

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)
The purpose is to study the interaction of calcitonin with its specific receptor target organs. The current investigations should provide further insight into the structure-function relationship in calcitonin. Calcitonin is a small polypeptide hormone and therefore lends itself well to studies using synthetic peptide fragments. The system is also useful for characterizing hormone receptors in kidney, bone and other tissues. Studies are in progress to characterize further the interaction of calcitonin with tissue receptors. It also will be of interest to solubilize the receptors and characterize them chemically.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 43004-17 MD

PERIOD COVERED
October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)
Studies on pseudohypoparathyroidism and related disorders

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

G. D. Aurbach, M.D., Chief, Metabolic Diseases Branch, NIADDKD
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A. M. Spiegel, M.D., Senior Investigator, MDB, NIADDKD
R. W. Downs, Jr., M.D., Senior Clinical Associate, MDB, NIADDKD
M. A. Levine, M.D., Medical Staff Fellow, Endocrinology & Genetics, MDB, NIADDK

COOPERATING UNITS (if any)

None

LAB/BRANCH
Metabolic Diseases Branch

SECTION

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Md. 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.00	.75	.25

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) In 1942 Albright and his associates described the features of a new clinical syndrome "pseudohypoparathyroidism" (PHP). Patients with this disorder differ from those with idiopathic hypoparathyroidism: they show characteristic constitutional features (Albright's hereditary osteodystrophy - AHO) and do not respond to exogenous parathyroid hormone. Subsequent to the original report, patients lacking the typical somatic features of AHO but resistant to endogenous and administered PTH have been described. We found previously that in PHP, U_{cAMP} (urinary cyclic AMP) does not increase normally in response to PTH administration. This indicated that there is a defective hormone receptor-adenylate cyclase complex in this disorder. We have now shown that red cells from patients with PHP + AHO are functionally deficient in a recently isolated protein ("G unit") that serves to couple hormone receptor to the catalytic unit of adenylate cyclase. Our results suggest that reduction in G units is the lesion responsible for hormone resistance in PHP + AHO and imply that the defect is not limited to PTH target organs (kidney and bone).

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 43005-17 MD

PERIOD COVERED

October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)

Hormone Receptors, Adenylate Cyclase and Cyclic AMP-Mediated Ion Transport

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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M. A. Levine, M.D., Medical Staff Fellow, Endocrinology & Genetics, MDB, NIADDK
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A. C. Santora II, M.D., Ph.D., Medical Staff Fellow, MDB, NIADDK

COOPERATING UNITS (if any)

None

LAB/BRANCH

Metabolic Diseases Branch

SECTION

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.00	2.25	1.75

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We have studied the components of the hormone receptor-adenylate cyclase complex. The turkey erythrocyte, a readily available, homogeneous cell type has been used as a model system. The turkey RBC plasma membrane possesses β-adrenergic receptors, identified using radioiodinated hydroxybenzylpindolol, a high-affinity β-blocker. β-receptors are coupled to adenylate cyclase catalytic units by a guanine nucleotide binding protein (G-unit) which we have isolated by affinity chromatography. We have studied the role of the G unit in mediating activation of adenylate cyclase by hormone, fluoride and cholera toxin. β-adrenergic agonist stimulation of cAMP accumulation in turkey erythrocytes leads to significant changes in sodium and potassium transport.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 43006-07 MD

PERIOD COVERED
October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)
Study of Hyperparathyroidism: Etiology, diagnosis and treatment.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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S. J. Marx, M.D., Senior Investigator, MDB, NIADDKD
A. M. Spiegel, M.D., Senior Investigator, MDB, NIADDKD
R. W. Downs, Jr., M.D., Senior Clinical Associate, MDB, NIADDKD
M. A. Levine, M.D., Medical Staff Fellow, Endocrinology & Metabolism, MDB, NIADDK
R. D. Lasker, M.D., Clinical Associate, MDB, NIADDKD
A. C. Santora, II, M.D., Ph.D., Medical Staff Fellow, MDB, NIADDK
J. L. Doppman, M.D., Radiology Dept., Clinical Center
Jeffrey Norton, M.D., Surgery Branch, NCI
J. T. Potts, Jr., M.D., Endocrine Unit, Massachusetts General Hospital
J. D. Gardner, M.D., Digestive Diseases Branch, NIADDKD
H. DeLuca, M.D., Department of Biochemistry, Univ. of Wisconsin

COOPERATING UNITS (if any)
Radiology Department, CC; Surgery Branch, NCI; Department of Biochemistry, University of Wisconsin; Endocrine Unit, MGH; Digestive Diseases Branch, NIADDKD

LAB/BRANCH
Metabolic Diseases Branch

SECTION

INSTITUTE AND LOCATION
NIADDKD, NIH, Bethesda, Md. 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.75	2.00	.75

CHECK APPROPRIATE BOX(ES)
☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The project goal is the evaluation and treatment of hyperparathyroidism. Patients with persistent postoperative hyperparathyroidism are referred for evaluation and treatment. Hereditary hyperparathyroidism in particular is under investigation in the hopes of delineating hereditary molecular abnormalities in glandular regulation, as exemplified in the multiple endocrine neoplasia syndromes. Evaluation ranges from epidemiologic studies of families to in-house studies of patients and to in vitro analyses of excised tissue. Techniques currently being employed and improved include radioimmunoassay of parathyroid hormone, selective arteriography and selective venous sampling for parathyroid hormone, parathyroid gland cryopreservation and autotransplantation, and transcatheter parathyroid gland infarction.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 43007-02 MD

PERIOD COVERED
October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)
Study of humoral hypercalcemia of malignancy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Allen M. Spiegel, M.D., Senior Investigator, MDB, NIADDKD
Leonard Deftos, M.D., University of California, San Diego
Dr. J. Heersche, St. Michael's Hospital, Toronto
R. W. Downs, Jr., M.D., Senior Clinical Associate, MDB, NIADDKD
S. J. Marx, M.D., Senior Investigator, MDB, NIADDKD
G. D. Aurbach, M.D., Chief, Metabolic Diseases Branch, NIADDKD
J. Norton, Surgery Branch, NCI
R. Fischer, NCI

COOPERATING UNITS (if any)

LAB/BRANCH
Metabolic Diseases Branch

SECTION

INSTITUTE AND LOCATION
NIADDKD, NIH, Bethesda, Md. 20205

TOTAL MANYEARS: 1.75	PROFESSIONAL: .75	OTHER: 1.00

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Hypercalcemia is a common and serious complication of cancer. In some patients, hypercalcemia occurs without direct tumor invasion of bone, presumably via a humoral mechanism. The identity of humoral factor(s) is not known, although PTH or prostaglandins may be the cause in some cases. The project goal is to identify factors responsible for humoral hypercalcemia of malignancy. This involves study of patients with this syndrome as well as an animal model for the syndrome, a Leydig cell tumor of Fischer rats.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AM 43008-01 MD

PERIOD COVERED
October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)

Vitamin D Resistance and Related Disorders

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

S. J. Marx, M.D., Senior Investigator, MDB, NIADDK
G. D. Aurbach, M.D., Chief, MDB, NIADDK
U. A. Liberman, M.D., Visiting Scientist, MDB, NIADDK
C. Eil, M.D., Bethesda Naval Medical Center

COOPERATING UNITS (if any)

LAB/BRANCH
Metabolic Diseases Branch

SECTION

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Md. 20205

TOTAL MANYEARS: 0.75	PROFESSIONAL: 0.75	OTHER: 0.00

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In 1937 Albright and co-workers suggested that rickets or osteomalacia might result from deficient action of vitamin D. With recognition that vitamin D is the precursor to $1,25(OH)_2D$, a steroidal hormone, it has become possible to identify patients with defective activation or target action of $1,25(OH)_2D$. We have shown that some patients with hereditary resistance to $1,25(OH)_2D$ have defective nuclear uptake of the hormone.

ANNUAL REPORT OF THE

CLINICAL ENDOCRINOLOGY BRANCH

The scientists of the Branch continued their broad approach to research on the hormones, their interactions with serum and cell proteins, their mechanism of action, and on endocrine diseases. The major achievements are given below under the headings of Thyroid Biochemistry, Polypeptide Hormones and Proteins of Cell Membranes. As in previous years, a number of scientists from abroad participated in this research, including individuals from France, India, Italy and South Africa.

I. Thyroid Biochemistry and Pathophysiology

A. Thyroid Cell Membrane and the Mechanism of TSH Action

In last year's report, evidence was presented that heterogeneous forms of TSH are found in TSH obtained from mouse thyrotropic tumor or from bovine pituitary. Those forms having the highest K_{av} - i.e., the lowest apparent molecular weight in chromatography on G-100 Sephadex-exhibited the lowest ratio of bioactivity to immunoactivity using adenyl cyclase activation in human thyroid membrane as the measure of biological action. However, these less active forms bound strongly to membrane receptors and were potent inhibitors of bovine TSH action. To determine possible carbohydrate differences between these heterogeneous forms, their interaction with specific lectins was examined by the technique of affinity chromatography. The apparent low-molecular weight, less active forms of TSH were found to contain decreased amounts (or availability) of α-mannose and increased amounts of β-N-acetylgalactosamine and/or β-galactose. These results confirm the earlier suggetion that variations in carbohydrate content are responsible for the microheterogeneity of both normal and tumor TSH and that this affects the biological activity of the molecule. (Weintraub, Joshi)

B. Thyroid Hormone Secretion

Further work has been done on the role of calcium ion in tubulin polymerization. The effect of Ca^{2+} to inhibit tubulin polymerization is dependent on a number of factors, including tubulin purity, temperature, and the presence of microtubule seeds or of microtubule-associated proteins (MAPs). In addition to intrinsic sensitivity to Ca^{2+}, there is a calmodulin-mediated sensitivity. The latter depends on MAPs; in their absence, Ca^{2+} promotes polymerization of 6S tubulin, whereas in their presence it is inhibited. Thus, regulation of microtubule assembly is complex. (Wolff; Lee)

The role of various agonists in thyroid stimulation has been extended to adenosine and forskolin. Both agents stimulate cAMP accumulation in thyroid pieces; however, adenosine is active only in a modified form, such as 5'-N-ethylcarboxamide adenosine, or N^6-phenyl iopropyl adenosine. These compounds are partial agonists when compared to TSH. (Wolff, Lee)

The stimulatory effect of forskolin, a diterpene, does not require GTP and is additive with Fl^-, GTP, and TSH. It is concluded that the adenosine molecules interact through an Ra type of membrane receptor whereas forskolin probably interacts via the G/F regulatory protein. (Wolff, Fradkin, Goldhammer)

C. Thyroxine-Protein Interactions

Further studies on the major thyroxine transport protein of human serum - thyroxine-binding globulin (TBG) - were performed to determine whether glycosylation might be responsible for the previously observed metastability and irreversible denaturation of this protein. TBG was deglycosylated by a mixture of bacterial glycosidases and its sensitivity to denaturation was determined by measuring tryptophan fluorescence in response to guanidinium chloride or mild acid. Deglycosylated TBG was found to be less stable than native TBG, and the resulting conformational change and loss of T_4-binding was irreversible. It was concluded that glycosylation of TBG is not responsible for the unusual stability properties of the native protein. (Robbins, Edelhoch, Grimaldi)

The effect of carbohydrate on the microheterogeneitiy of TBG was also studied. Intact TBG gives four or more protein bands after isoelectric focussing. After removal of 85% of the carbohydrate residues, a residual microheterogeneity consisting of two bands was found. This suggests that a polymorphism of the polypeptide chain of TBG may exist. Since pooled serum was used in these analyses, the study is being extended to individual sera to further evaluate this possibility. (Robbins, Grimaldi, Ramacciotti)

Controlled photolysis of thyroxine in the near UV was previously shown to yield 3',3,5-triiodothyronine. When this reaction was carried out with radioactive T_4 in the presence of human serum, or thyroxine-binding proteins isolated from serum, it was found that TBG and albumin were covalently labeled in the T_4-binding site, whereas PA was not. The extent of labeling of the binding site by the free radical of monodeiodinated T_4 evidently depends on the nature of the amino acid residues which are in close proximity to the hormone. This procedure promises to be useful in defining the nature of the binding sites on TBG and albumin. (Cahnmann, van der Walt)

D. Thyroid Hormone Metabolism

Continuous monolayer cultures of monkey hepatoma cells and primary monolayer cultures of normal rat hepatocytes have been used as model systems in which to study the metabolism of the thyroid hormones. Since the primary step in metabolism is the entry of hormone into the cell, this work is being extended by developing methods to measure the rate of uptake of hormone by cells. A technique employing suspended cells and their sedimentation through a layer of oil in a microcentrifuge permits rapid separation of cells from the incubation medium at time points as early as 2 seconds. The experiments thus far have been limited to the monkey hepatoma cells, which show uptake of T_3 at concentrations as low as 0.5×10^{-12} M only by a nonsaturable process. This is at variance with work in other laboratories with normal hepatocytes which have reported up to three saturable processes and suggests that the hepatoma cells may be abnormal in this respect. (Robbins, Movius)

E. Thyroid Hormone Action

A major site of action of thyroid hormones is in the cell nucleus that involves the interaction of T_3 with a specific protein receptor. The photoaffinity labeling technique described in Section IC has been used to study the receptor. Three proteins were covalently labeled and were shown to have apparent molecular weights of 56,000, 45,000 and 12,000 by SDS-gel electrophoresis. A similar labeling pattern was observed after affinity labeling with N-bromoacetyl-T_4 or T_3. Studies are in progress to establish the specificity of these proteins for hormone binding and to characterize them further. (Cahnmann, Nikodem)

Chromatin from hepatic nuclei of thyroidectomized rats was less easily solubilized by nuclease than normal chromatin. T_3 treatment gave a detectable increase in 4.5 hours. Thus, thyroid hormone treatment causes changes in nuclear proteins which alter the structure of chromatin in such a way as to expose more DNA to nuclease attack. (Rall, Nikodem)

F. Studies in Thyroid Disease

The evaluation of low dose radioiodine ablation therapy (30 mCi ^{131}I) after thyroidectomy for thyroid cancer has been completed in 20 patients. Total ablation was achieved in 40% of patients after a single dose and in 60% after two doses. Low dose therapy, which does not require hospitalization, is therefore indicated in young subjects in whom it is especially important to minimize unwanted whole body radiation. In older patients, or in those with aggressive cancers, therapy with 100 mCi is preferable. (Robbins, Pretorius, Ramacciotti)

Further studies have been carried out on the syndrome of inappropriate secretion of TSH. One patient who has been followed at NIH for 18 years has recently shown decreasing peripheral resistance to thyroid hormone while her pituitary resistance has remained unchanged. Since administration of T_4 or T_3 to reduce the TSH-induced goiter results in hyperthyroidism, bromergocriptine has been tried as a means to suppress TSH by central action. Preliminary results suggest this to be effective therapy in one of two patients so treated.

Two new families with the syndrome have been identified, and a total of 8 affected members have been found thus far. As shown previously in isolated cases, the familial subjects have normal nuclear T_3 receptors in skin fibroblasts. (Weintraub, Magner)

Follow-up of 9 women with biopsy-proved postpartum lymphocytic thyroiditis has revealed chronic thyroiditis in three, and an exacerbation not associated with pregnancy in one. It seems clear that these patients have a latent autoimmune process and that the postpartum period is just one of several factors that can activate the disease. (Weintraub, Magner)

II. Polypeptide Hormones

A. Biosynthesis of Glycoprotein Hormones

Previous studies have shown that the asparagine-linked carbohydrate chains may play critical roles in TSH biosynthesis. The initial steps in glycopeptide synthesis have now been investigated. Lipid-linked polysaccharide units are initially transferred to both α and β subunits, followed by rapid removal of two glucose residues. Removal of the third glucose and several mannose residues proceeds more slowly. Final conversion to endoglycosidase-H resistant glycopeptide forms depends on the nature of the subunit and occurs more rapidly in β-TSH. (Weintraub, Ronin)

Further work has been done to define the subcellular events in the biosynthesis of TSH. Mouse pituitary tumor was used in pulse-chase experiments with ^{35}S-Met or ^{3}H-mannose. The rate of passage from rough to smooth endoplasmic reticulum was faster for β-subunit than for α, but the rate was fastest when α and β were combined in TSH. Processing of the carbohydrate chains begins in the RER and continues in the SER and Golgi fractions, where the branched chain ("complex") oligosaccharide units are formed. Coupling of α and β subunits also is not complete until the components reach the SER and Golgi. (Weintraub, Magner, Ronin)

The biosynthesis of hCG in cultured choriocarcinoma cells was investigated further. As a tool in this work, antibodies were developed which differ in their reaction with hCG depending on the state of combination of the subunits. An anti-β serum reacted both with free and combined β, whereas anti-α-serum reacted only with the free subunit. Labeled carbohydrate incorporation experiments have shown that the free α contains more fucose and sialic acid than the smaller, combined α subunit. Inhibition of glycosylation with tunicamycin did not prevent secretion of α and β subunits into the culture medium. However, only glycosylated species were found combined in hCG. (Weintraub, Rosen, Jones-Brown)

Ectopic polypeptide hormone biosynthesis is under study in cultured Chang liver cells which produce the common α subunit. It has been found that ^{35}S-Met is incorporated into an α form which has a slightly higher apparent molecular weight than eutopic hCG-α. ^{3}H-carbohydrates are incorporated into forms having both higher and lower molecular weight, and it has been found that butyrate tends to increase glycosylation whereas dibutyryl cyclic AMP inhibits glycosylation. Fucose incorporation into ectopic hCG has also been shown, and is contrary to reports from other laboratories with eutopic hCG. (Rosen, Weintraub, Morrow)

B. Ectopic Polypeptide Hormone Syndromes

In vitro studies have been carried out on several different tumor markers. In HeLa/fibroblast human cell hybrids, both α subunit and placental alkaline phosphatase isoenzyme are expressed. Both of these substances have been used as clinical markers of malignancy. Interestingly, the α subunit correlated with malignancy as tested in athymic "nude" mice. It has also been shown that a "placental-specific" protein PP_5, is synthesized by normal human fibroblasts and that its rate of secretion into the culture medium is different from SP_1, another "placental-specific" tumor marker. Moreover, in a fibrosarcoma cell line (A1632), SP_1 is synthesized but PP_5 is not. (Rosen)

Several additional clinical studies on placental and glycoprotein hormones as potential tumor markers have been performed. In 51 patients with multiple myeloma and 58 others with monoclonal or other gammapathies, measurement of α and β subunits of chorionic gonadotropin, placental lactogen, and SP_1 showed that none of these placental proteins would be useful in distinguishing benign from malignant plasma cell dyscrasias. Only α subunit was elevated in a small number of patients.

Contrary to an earlier report that SP_1 is elevated in breast carcinoma, its measurement in 191 such patients compared with 27 normal women and 33 with benign breast disease revealed no significant differences between the groups. In a small series of uremic patients, however, SP_1 was elevated in about 30 per cent.

Preliminary results in patients with choriocarcinoma or ectopic placental lactogen (PL) secretion indicate the presence of high molecular weight PL similar to that found in the culture medium of normal placental explants or choriocarcinoma cells. (Rosen, Weintraub, Morrow)

C. Gonadotropin Pathophysiology

A study of the episodic release of gonadotropins in patients with various forms of hypogonadotropic hypogonadism and in anosmic relatives showed that only the eugonadal relatives had LH or FSH pulses. The number of these occurring per day were probably normal, although more data are needed to be certain of this. (Rosen, Morrow)

III. Studies on Protein Structure and on the Interactions of Proteins with Cell Membranes

In further studies on the coated vesicles of the cell surface, it has been possible to obtain preparations which sediment as a single boundary on sucrose gradients and have a constant ratio of protein to phospholipid throughout the boundary. The protein (clathrin) and phospholipid (membrane) can be dissociated at pH 8.5 in 0.01 M Tris buffer, and they reassociate to form native coated vesicles only if they are first adjusted to pH 7.5 and then the pH is lowered to 6.5. Since the conditions required for the reformation of coated vesicles are similar to those required for self association of clathrin, it appears that clathrin-clathrin interactions control the rate and extent of coated vesicle formation. The influence of Ca^{2+} is of interest since in its presence the vesicle can be coated at physiological pH. By regulating the Ca^{2+} concentration, the coat can either be removed or replaced. (Edelhoch, Nandi, Prasad, Alfsen)

IV. Mechanism of Hormone Action

Knowledge of the role of Ca^{2+}, calmodulin and hydrophobic interactions in the activation of Bordetella pertussis adneylate cyclase has been extended. The cyclase in crude bacterial membrane preparations could be activated by low levels of calmodulin, with a dose-dependent response occurring between 2 picograms and 100 nanograms. This provides an excellent bioassay for measuring calmodulin levels in a variety of protein and cellular preparations.

The effects of amphophiles, such as phospholipids and nonionic detergents, were studied primarily on the soluble adenylate cyclase of B. pertussis culture medium. Stimulation by these substances affects the Vmax and not the Km of the enzyme and gangliosides were shown to be inhibitory. By studying a number of compounds, it was shown that the stimulation was dependent on high-affinity hydrophobic binding sites and that it requires Ca^{2+} and is sensitized by calmodulin. It appears that the amphophile effects may mimic the hydrophobic interactions related to Ca^{2+}-calmodulin activation of the enzyme. Since EGTA does not prevent stimulation, calmodulin itself may be considered as the activator. (Wolff, Goldhammer, Kilhoffer)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 45000-15 CEB

PERIOD COVERED
October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Thyroxine Protein Interactions

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. Robbins	Chief, Clin. Endo. Br.	CEB NIADDK
Other:	H. Edelhoch	Chief, Prot. Struct. Sec.	CEB NIADDK
	S. Grimaldi	Visiting Fellow	CEB NIADDK
	C. Ramacciotti	Guest Worker	CEB NIADDK
	M. Phyillaier	Biologist	CEB NIADDK

COOPERATING UNITS (if any)

None

LAB/BRANCH
Clinical Endocrinology Branch

SECTION
Endocrine Physiology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.9	1.5	.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The effect of the carbohydrate chains on the susceptibility of thyroxine-binding globulin (TBG) to denaturation was evaluated. Removal of 85% of the carbohydrate decreased the stability of the protein to guanidinium chloride or to acid. The initial structural change was irreversible, indicating that incorporation of carbohydrate during biosynthesis of TBG is not responsible for the metastability of the protein. Deglycosylated TBG was also analyzed by isoelectric focussing, which revealed the presence of two protein bands as seen with desialylated TBG. This microheterogeneity therefore appears to reside in the polypeptide chain.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 45003-08 CEB

PERIOD COVERED
October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Synthesis of Thyroid Hormone Residues in Thyroglobulin (formerly Model Reactions for the Synthesis of Thyroxine Residues in Thyroglobulin)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. J. Cahnmann	Scientist Emeritus	CEB NIADDK
Other:	J.-M. Gavaret	Charge de Recherche	Coop Unit (see below)
	J. Nunez	Chief	Coop Unit (see below)

COOPERATING UNITS (if any)
Unité de Recherche sur la Glande Thyroide et la Régulation Hormonale, INSERM, Bicetre, France

LAB/BRANCH
Clinical Endocrinology Branch

SECTION
Endocrine Biochemistry

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project has been continued by Cooperating Unit in France without participation of NIH.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 45004-11 CEB

PERIOD COVERED
October 1, 1981, to September 30, 1982

TITLE OF PROJECT (30 characters or less)

Structure of Polypeptide and Protein Hormones

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. Edelhoch	Senior Scientist	CEB NIADDK
Other:	P. Nandi	Visiting Scientist	CEB NIADDK
	K. Prasad	Visiting Fellow	CEB NIADDK
	A. Alfsen	Visiting Scientist	CEB NIADDK
	J. Robbins	Chief, Clin. Endo. Br.	CEB NIADDK
	A. Minton	Research Chemist	LBP NIADDK
	S. Grimaldi	Visiting Fellow	CEB NIADDK
	R. E. Lippoldt	Sr. Health Services Officer	CEB NIADDK

COOPERATING UNITS (if any)

None

LAB/BRANCH
Clinical Endocrinology Branch

SECTION
Protein Structure

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 5.4	PROFESSIONAL: 4.2	OTHER: 1.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Various molecular and chemical properties of homogenous preparations of coated vesicles have been determined.

The conditions required to dissociate coated vesicles into protein and phospholipid components have been determined. Under certain conditions, the dissociation may be reversed to give a good yield of coated vesicles with the properties of the native structure.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM-45009-15 CEB

PERIOD COVERED
October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Studies in Thyroid Diseases

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. Robbins	Chief, Clin. Endo. Br.	CEB NIADDK
Other:	B. D. Weintraub	Medical Director	CEB NIADDK
	C. Ramacciotti	Guest Worker	CEB NIADDK
	J. Magner	Clinical Associate	CEB NIADDK
	J. Fradkin	Clinical Associate	CEB NIADDK
	E. Movius	Medical Staff Fellow	CEB NIADDK
	A. Keenan	Staff Fellow	NM CC
	S. H. Barsky	Expert	LP NCI
	E. W. Chu	Head, Cytopath. Sec.	LP NCI

COOPERATING UNITS (if any)

Nuclear Medicine Department, CC; Laboratory of Pathology, NCI

LAB/BRANCH
Clinical Endocrinology Branch

SECTION
Endocrine Physiology Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.3	2.1	.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In the treatment of thyroid cancer with radioactive iodine, it has been shown that low-dose ablation therapy (30 mCi ^{131}I) is useful in subjects under age 40 years since 40% experience total ablation after one dose and at least 60% after two doses. Older subjects or those with aggressive cancer should receive 100 mCi ^{131}I in order to achieve ablation more quickly. Additional patients with the syndromes of inappropriate secretion of TSH and postpartum thyroiditis have been identified and are being studied to determine the pathogenesis of these disorders.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 45010-12 CEB

PERIOD COVERED
October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Ectopic Protein and Subunit Production by Tumors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. W. Rosen	Medical Director	CEB NIADDK
Other:	B. D. Weintraub	Medical Director	CEB NIADDK
	J. S. Morrow	Clinical Associate	CEB NIADDK
	J. Osborne	Sr. Investigator	IR MD NHLBI
	E. Heyderman	Sr. Lecturer, Dept. Path.	St. Thomas's Hosp., London
	D. Tormey	Assoc. Prof., Dept. Med.	U. Wisc. Med. Ctr.
	C. Horne	Prof., Dept. Path.	U. Aberdeen, Scotland
	R. A. Kyle	Staff Physician	Mayo Clinic
	J. Solano	Director	Mid-Atlantic Nephrology Center
	E. Stanbridge	Prof., Dept. Microbiol.	U. Calif., Irvine
	H. Sussman	Prof., Dept. Path.	Stanford

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Clinical Endocrinology

SECTION
Endocrine Physiology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	.9	1.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We continue to study placental proteins as cancer markers. Ectopic production of alpha subunit *in vitro* by Chang liver cells is differentially regulated by butyrate and by dibutyryl cyclic AMP. Alpha production *in vitro* by HeLa-fibroblast human cell hybrids is specifically correlated with malignancy but production of placental alkaline phosphatase showed no such correlation. Partially deglycosylated SP_1 (79% carbohydrate removed) has an apparent molecular weight of 30,500. Placental protein 5 production by fibroblasts is regulated differently from SP_1 production by these cells.

Placental proteins are not useful in distinguishing malignant from benign plasma cell dyscrasias or malignant from benign breast lesions.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 45013-10 CEB

PERIOD COVERED
October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Biosynthesis and Glycosylation of Thyrotropin (formerly Biosynthesis of Glycoprotein Hormones)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. D. Weintraub	Medical Director	CEB NIADDK
Other:	B. S. Stannard	Biologist	CEB NIADDK
	J. Magner	Clinical Associate	CEB NIADDK
	C. Ronin	Visiting Fellow	CEB NIADDK

COOPERATING UNITS (if any)

None

LAB/BRANCH
Clinical Endocrinology Branch

SECTION
Endocrine Physiology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	1.8	1.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Thyrotropin (TSH) in mouse thyrotropic tumor cells is glycosylated through a dolichol lipid-linked oligosaccharide containing 3 glucoses, 9 mannoses and 2 N-acetylglucosamines. The first α glucoses on subunits are rapidly removed within 10 minutes but the third is removed more slowly. Subsequently over the next two hours, the last glucose and 6 of the 9 mannoses are removed and additional sugars added to produce complex oligosaccharides; β is processed more rapidly than α. Subcellular fractionation studies indicate that the initial steps in carbohydrate processing occur in the rough endoplasmic reticulum, but the late addition of sugars occurs in the Golgi and post-Golgi compartments.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 45014-11 CEB

PERIOD COVERED
October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Thyroid Membranes and Secretion (formerly Adrenal and Thyroid Secretion)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. Wolff	Medical Director	CEB NIADDK
Other:	J. Fradkin	Clinical Associate	CEB NIADDK
	A. Goldhammer	Staff Fellow	CEB NIADDK
	G. H. Cook	Chemist	CEB NIADDK

COOPERATING UNITS (if any)

C. Londos and D. M. F. Cooper, LNE, NIADDK

LAB/BRANCH
Clinical Endocrinology Branch

SECTION
Endocrine Biochemistry

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	.6	.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The role of various agonists in thyroid stimulation has been extended to adenosine and forskolin. Both agents stimulate cAMP accumulation in guinea pig thyroid pieces but adenosine and its analogues are partial agonists. They interact through an Ra type of membrane receptor, whereas forskolin probably interacts via the G/F regulatory protein.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 45015-12 CEB

PERIOD COVERED
October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Thyroid Plasma Membranes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. Wolff	Medical Director	CEB NIADDK
Other:	J. Fradkin	Clinical Associate	CEB NIADDK
	A. Goldhammer	Staff Fellow	CEB NIADDK
	G. H. Cook	Chemist	CEB NIADDK

COOPERATING UNITS (if any)

C. Londos and D. M. F. Cooper, LNE, NIADDK

LAB/BRANCH
Clinical Endocrinology Branch

SECTION
Endocrine Biochemistry

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 1.7	PROFESSIONAL: 1.1	OTHER: .6

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Combined with Project Number Z01 AM 45014-11

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 45016-12 CEB

PERIOD COVERED
October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Thyroid Hormone Secretion and the Function of Microtubules (formerly Thyroid Hormone Secretion)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. Wolff	Medical Director	CEB NIADDK
Other:	Y. Lee	Staff Fellow	CEB NIADDK

COOPERATING UNITS (if any)

None

LAB/BRANCH
Clinical Endocrinology Branch

SECTION
Endocrine Biochemistry

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 1.4	PROFESSIONAL: 1.2	OTHER: .2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The role of Ca^{2+} in tubulin polymerization has been further investigated. Ca^{2+} interacts with pure 6S tubulin and systems containing microtubule associated proteins (MAPs) as well. The effect is intrinsic to these preparations but is sensitized by the presence of calmodulin. The latter protein requires the presence of MAPs to inhibit polymerization and current efforts are directed toward identifying which of the associated proteins is the primary regulator.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 45018-07 CEB

PERIOD COVERED
October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Adenylate Cyclase and Other Extracellular Products of B. Pertussis: Effects on Mammalian Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. Wolff	Medical Director	CEB NIADDK
Other:	A. Goldhammer	Staff Fellow	CEB NIADDK
	H. G. Cook	Chemist	CEB NIADDK
	M.-C. Kilhoffer	Visiting Fellow	CEB NIADDK

COOPERATING UNITS (if any)
None

LAB/BRANCH
Clinical Endocrinology Branch

SECTION
Endocrine Biochemistry

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	.9	.6

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER
☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The role of Ca^{2+}, calmodulin and hydrophobic interactions in the activation of *Bordetella pertussis* adenylate cyclase has been extended. A stable spheroplast membrane preparation has been developed for a very sensitive assay procedure to as little as < 1 femtomole of the regulator protein. Hydrophobic interactions are shown by 3-10-fold stimulation by various amphiphiles that is not dependent on micelle formation but does depend on Ca^{2+}. Nevertheless, we have now found conditions where we can demonstrate a Ca^{2+}-independent activation of the *B. pertussis* adenylate cyclase by calmodulin and the basis of this interaction is under study.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 45020-06 CEB

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Synthesis of Thyroxine Transport Proteins

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. Robbins	Chief, Clin. Endo. Br.	CEB NIADDK
Other:	L. Bartalena	Fogarty International Fellow	CEB NIADDK
	M. Phyillaier	Biologist	CEB NIADDK

COOPERATING UNITS (if any)

VA Hospital, Washington, D. C.; University of Brussels, Belgium

LAB/BRANCH
Clinical Endocrinology Branch

SECTION
Endocrine Physiology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 1.4	PROFESSIONAL: 1.0	OTHER: .4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A continuous culture of monkey hepatoma cells has been shown to still secrete thyroxine-binding globulin (TBG), seven years after the initial demonstration in our laboratory. Studies are under way to define the intracellular steps in peptide chain and carbohydrate chain synthesis of this protein.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 45021-06 CEB

PERIOD COVERED
October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Clinical Pathophysiology of Pituitary Gonadotropins

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. W. Rosen	Medical Director	CEB NIADDK
Other:	J. M. Lieblich	Director, Med. Educ.	Highland Park Hosp., IL
	A. S. Rogol	Assoc. Prof. Ped.	Univ. Va. Sch. of Med., VA
	B. White	Sr. Investigator	LEP NIADDK
	J. Morrow	Clinical Associate	CEB NIADDK

COOPERATING UNITS (if any)

Highland Park Hospital, Department of Medicine, IL; University of Virginia School of Medicine, VA

LAB/BRANCH
Clinical Endocrinology Branch

SECTION
Endocrine Physiology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.6	.4	.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Studies continue on syndromes of hypogonadotropic hypogonadism with anosmia (AHH, the Kallmann syndrome) and without anosmia (HH), as well as on patients with anosmia who are clinically eugonadal. Episodic secretion of pituitary gonadotropins has been in monitored eight patients with AHH, six with HH and three with anosmia alone. The AHH and HH patients with one exception, had no LH pulses; the anosmics had several LH pulses in 24 hours.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 45023-06 CEB

PERIOD COVERED
October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Thyrotropin Receptor-Binding and Action: Relationship to Anti-thyroid Immunoglobulins

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. D. Weintraub	Medical Director	CEB NIADDK
Other:	L. Joshi	Visiting Fellow	CEB NIADDK

COOPERATING UNITS (if any)

None

LAB/BRANCH
Clinical Endocrinology Branch

SECTION
Endocrine Physiology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 1.4	PROFESSIONAL: 1.2	OTHER: .2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Heterogeneous forms of bovine and mouse TSH show differences in their bioactivity (B/I) ratio. Examination of the interaction between certain forms of mouse tumor or bovine pituitary TSH with standard bTSH revealed that those forms with low B/I act as competitive antagonists to more bioactive forms. Lectin-binding studies suggest that there are differences in carbohydrate content between the TSH with high biologic activity and that with lower activity.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 45024-06 CEB

PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Metabolism of Thyroid Hormones

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. Robbins	Chief, Clin. Endo. Br.	CEB NIADDK
Other:	M. Phyillaier	Biologist	CEB NIADDK

COOPERATING UNITS (if any)

None

LAB/BRANCH

Clinical Endocrinology Branch

SECTION

Endocrine Physiology

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0	PROFESSIONAL: 0	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Inactive

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 45025-04 CEB

PERIOD COVERED
October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

The Effects of Triiodothyronine on Liver Nucleoproteins

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. E. Rall	Director, Intramural Research Programs	ODIRP NIADDK
Other:	V. M. Nikodem	Staff Fellow	CEB NIADDK
	H. Cahnmann	Scientist Emeritus	CEB NIADDK
	B. van der Walt	Visiting Fellow	CEB NIADDK

COOPERATING UNITS (if any)

None

LAB/BRANCH
Clinical Endocrinology Branch

SECTION
Protein Structure

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.4	1.3	.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Differences in the rate of digestion of DNA rat liver nuclei of control, thyroidectomized and T_3-treated thyroidectomized rats revealed that chromatin from thyroidectomized liver nuclei was less easily solubilized by nuclease than chromatin from normal liver nuclei. It is suggested that thyroid hormone causes changes in nucleoproteins which alter the structure of chromatin in such a way as to expose more DNA to nuclease attack and/or increase the solubility of released nucleosomes. To study the mechanism of action of thyroid hormones, polyadenylated RNA must be prepared and specific antibody stimulated. Malic enzyme from fully stimulated rats was purified by affinity chromatography. This material was used to stimulate goat antibody. Total RNA, containing polyadenylated RNA, was prepared from the same group of rats as malic enzyme. This RNA translated proteins of different sizes and malic enzyme was immunologically precipitated using prepared antibody. Underivatized thyroid hormones were used for a covalent labeling of rat liver nuclear proteins. Results show the presence of a wide range of nuclear proteins which can be labeled with 1 nM of ^{125}I-T_4. The proteins which responded best were those having a molecular weight of 96,000, 56,000, 45,000 and 12-15,000.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 45028-04 CEB

PERIOD COVERED
October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Thyroid Hormones-Cell Interactions

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. Robbins	Chief, Clin. Endo. Br.	CEB NIADDK
Other:	E. Movius	Medical Staff Fellow	CEB NIADDK
	M. Phyillaier	Biologist	CEB NIADDK

COOPERATING UNITS (if any)

None

LAB/BRANCH
Clinical Endocrinology Branch

SECTION
Endocrine Biochemistry

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.4	.8	.6

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Preliminary experiments have been completed on the uptake of triiodothyronine by cultured monkey hepatoma cells. A method allowing uptake measurement in less than 2 seconds has failed to show a saturable uptake process, as observed by others in normal rat hepatocytes.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 45029-03 CEB

PERIOD COVERED
October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

POEMS Syndrome (formerly Hyperpigmentation Gammopathy Syndrome)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. W. Rosen	Medical Director	CEB NIADDK
Other:	J. Morrow	Clinical Associate	CEB NIADDK

COOPERATING UNITS (if any)

None

LAB/BRANCH
Clinical Endocrinology Branch

SECTION
Endocrine Physiology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: .4	PROFESSIONAL: .3	OTHER: .1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Inactive

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 45030-02 CEB

PERIOD COVERED
October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Biosynthesis and Glycosylation of Human Chorionic Gonadotropin

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. D. Weintraub	Medical Director	CEB NIADDK
Other:	S. W. Rosen	Medical Director	CEB NIADDK
	Y. Jones-Brown	Staff Fellow	CEB NIADDK
	H. G. Fein	Staff Physician	Walter Reed Army Med. Ctr.

COOPERATING UNITS (if any)

Walter Reed Army Medical Center, Washington, D. C.

LAB/BRANCH
Clinical Endocrinology Branch

SECTION
Endocrine Physiology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.6	1.4	.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Biosynthesis of hcG subunits in choriocarcinoma cells has been studied by incorporation of labeled carbohydrate precursors combined with gel filtration of glycopeptides obtained by pronase digestion of immunoprecipitated subunits before and after exoglycosidase treatment. The large molecular weight free hCG-α contained more labeled fucose and sialic acid than the smaller combined α. Inhibition of glycosylation with tunicamycin impaired subunit combination but not secretion.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 45031-02 CEB

PERIOD COVERED
October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Photolysis of Thyroxine and Thyroxine Analogs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Ben van der Walt	Visiting Fellow	CEB NIADDK
Other:	Hans J. Cahnmann	Scientist Emeritus	CEB NIADDK

COOPERATING UNITS (if any)

None

LAB/BRANCH
Clinical Endocrinology Branch

SECTION
Endocrine Biochemistry

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.7	.5	.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The synthesis of [3-^{125}I],3',5'-triiodo-L-thyronine (inner ring-labeled rT_3) of high specific activity ("carrier-free") has been perfected.

The photolysis of thyroxine and thyroxine analogs has been modified to include the synthesis of metabolites of thyroxine on a large scale.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM-45032-01 CEB

PERIOD COVERED
October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Photoaffinity Labeling of Binding Proteins for Thyroid Hormones

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	V. M. Nikodem	Staff Fellow	CEB NIADDK
Other:	B. van der Walt	Visiting Fellow	CEB NIADDK
	H. J. Cahnmann	Scientist Emeritus	CEB NIADDK
	J. E. Rall	Scientific Director	IRP NIADDK

COOPERATING UNITS (if any)

None

LAB/BRANCH
Clinical Endocrinology Branch

SECTION
Protein Structure Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: .9	PROFESSIONAL: .8	OTHER: .1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Photoaffinity labeling of human serum albumin, thyroxine-binding globulin, prealbumin or rat liver nuclear proteins (NE) or whole nuclei (N) with L-thyroxine or 3,5,3'-triiodo-L-thyronine was achieved by irradiation in the near U.V. The major nuclear proteins that were covalently labeled have Mr of about 56,000, 45,000 and 12,000, respectively. Similar labeling patterns were observed with 3,3',5'-triiodo-L-thyronine (rT_3) and with N-bromo-acetyl-T_3 (or $-T_4$) (without irradiation).

PHS-6040
(Rev. 2-81)

ANNUAL REPORT 1981 - 1982
Diabetes Branch, NIADDK

Recognition of Previous Achievements

Alumni of the Diabetes Branch continue to prosper. One of our alumni received the Ernst Oppenheimer Memorial Prize of the Endocrine Society, which annually recognizes a distinguished young investigator. Another alumnus was elected to the American Society for Clinical Investigation. Another alumnus received his tenure appointment at the International Institute for Cellular and Molecular Pathology in Brussels.

Grants were made to members of the Diabetes Branch from both the Juvenile Diabetes Foundation and the American Diabetes Association. In addition, the Diabetes Branch members received recognition from the American Academy of Arts and Sciences; the Harvey Society; Banting Medal of the American Diabetes Association for scientific achievement; and the Mellon Lectureship of the University of Pittsburgh.

Insulin and Related Hormones

Evolutionary Origins of Vertebrate Hormones -- In previous work, we showed insulin was present in unicellular protozoa (eukaryotes) and bacteria (prokaryotes). In addition, ACTH, beta endorphin, and somatostatin were found in protozoa. These materials were further characterized. Also we demonstrated insulin in additional strains of E. coli (bacterium) and in additional strains of tetrahymena (protozoa) and the time course of their production was studied. ACTH was demonstrated in one more strain of protozoa and in E. coli. In addition, studies on relaxin, calcitonin, CCK-PZ, and vasotocin have been extended while the work on dynorphin and glucagon has been temporarily halted.

Ontogeny of Insulin-- In line with other studies to show that insulin can be produced in the absence of pancreatic beta cells, we showed that insulin was present in the chick at 2-3 days of embryogenesis before beta cells or pancreatic insulin were recognizable (days 3-1/2 to 4). The material was indistinguishable from avian insulin but distinctly different from mammilian insulin. Unfertilized egg yolk and egg white also contain small concentrations of material very similar to avian insulin.

Biosynthesis of Insulin Receptors -- In the last several years numbers of the Branch developed techniques to label the insulin receptor with both carbohydrates and proteins, both intrinsically and extrinsically. In the present study, a 200K receptor-related cellular protein was labeled with mannose, glucosamine and methionine; this protein appears to be the precursor for several other receptor-related proteins, including the two well established subunits of 135K and 95K as well as a less well studied 210K material. The 200K precursor is present only inside the cells and appears early; in a time-dependent way, it yields 135K and 95K material as well as the 210K protein, all three of which were found in the plasma membrane as well as in the cell interior. In summary, an early intracellular precursor of the cell surface receptor has been detected which appears to have a small number of carbohydrate residues (i.e. core carbohydrate) which then undergoes changes in size including further addition of carbohydrates to the proteins. This approach is now being applied to study cells in culture from patients with receptor-related disorders.

Extreme Insulin Resistance -- Some patients with extreme insulin resistance have congenital defects at the level of insulin binding to its receptor or at other early steps of insulin action. To study this problem we examined fibroblasts cultured from one patient with a severe alteration in the number of insulin receptors *in vivo*. These cells showed a decrease in the affinity of their insulin receptors, a decrease in the number of EGF receptors, and an alteration in the response of the receptor to changes in the levels of glucose, EGF and insulin in the medium.

Another patient has cells with a normal number of insulin receptors but the affinity of these receptors for insulin is quite insensitive to changes in pH and temperature. Further, this patient's receptors show major alterations in one of the cooperativity sites of the receptor, which regulates the rate of dissociation of insulin from the insulin receptor. Although this receptor has an extremely rare defect, it provides an exceptional opportunity to study cooperativity and the regulation of receptor affinity. Equally important it appears that the transmission of the biological message from the receptor to the cell is associated with alterations in receptor affinity. This mutant provides an exceptional opportunity to study this relationship.

In Vitro Correlates of Insulin Binding to Receptor In Vivo -- Glucocorticoids, which are an important modulator of glucose metabolism *in vivo*, produced *in vitro* an increase in receptor concentration due to induction of insulin receptors. Simultaneously there was a fall in growth hormone receptors. In short term studies *in vivo*, there was no effect demonstrated on the insulin receptor. These studies provide new insights into the mechanism of action of glucocorticoids on glucose homeostasis.

In related studies, attempts were made to demonstrate effects of sulfonylureas on insulin binding *in vitro*. No effect was demonstrated. Likewise, it was shown that sulfonylureas in insulin-dependent diabetics produced no changes, refocusing attention on the beta cell at the major site of sulfonylurea action.

Insulin receptors on red cells have been studied in several circumstances. While red cells, like monocytes, tend to reflect disorders of glucose metabolism, correlations in individual patients between binding on red cells and binding on white cells are not close. In addition, patients with insulin-dependent diabetes mellitus who show decreased insulin binding to monocytes, appear to show no alteration in insulin binding to red cells. Further studies of insulin binding to its receptor in these model systems should provide better insights into mechanisms of insulin sensitivity *in vivo*.

Morphological Correlates -- Morphological correlates (at the cellular and subcellular level) of hormone binding to cell surface receptors has been a major focus of the Branch. Recent studies have shown that radioactively labeled human growth hormone shows an unexpectedly high fraction of irreversible binding both at the surface and in internalized regions. Inhibition of lysosomal enzymes increased binding, probably by inhibiting degradation of receptor-bound hormone. Furthermore, it has been shown that hormone-induced down regulation is associated with internalization of receptor that has hormone bound to it.

Insulin binding to the cell surface appears initially to be random followed by relative concentration at the Golgi end of the cell. At a further level of discrimination it has been found that the surface of the microvilli of plasma membrane bind more insulin; hormone binding is followed by movement of hormone-occupied receptors to the nonvillous regions of the cell surface.

When insulin receptors are labeled directly and then treated with insulin, the labeled insulin-treated receptors are preferentially internalized. Also it is possible to show that there is reversal of the orientation of these receptors.

Glucagon binding to its receptors has been shown to resemble strongly insulin binding to its receptors except that the internalization appears to be slower with more of the hormone-receptor complexes staying on the cell surface. Along with the slower rate of internalization is a slower rate of hormone degradation.

Receptor Phosphorylation -- Insulins binds to its receptor and thereby activates the receptor; the activated receptor then activates processes in the cell associated with insulin action. One of the earliest events triggered by insulin binding to its receptor is phosphorylation of the receptor, largely in tyrosine residues, which is like the phosphorylations found in response to transforming agents as well as messenger molecules such as epidermal growth factor, platelet-derived growth factor and acetylcholine. In intact cells, the 95K subunit of the insulin receptor is phosphorylated. In more recent studies, plasma membranes were isolated and solubilized, and the receptor was purified by affinity chromatography on lectin columns. The addition of insulin to the solubilized purified insulin receptor resulted in a prompt phosphorylation of both subunits with the 95K subunit showing about 5 times more phosphorylation than the 135K. The response showed the specificity typical of metabolic actions of insulin, was rapid (minutes) and sensitive to insulin at 3×10^{-10}M. Thus, insulin-stimulated phosphorylation of the insulin receptor appears to be an early event in insulin action and all the components needed are contained in the plasma membrane.

Acromegaly -- As part of an ongoing study of acromegaly, we found that the concentration of growth hormone in plasma was directly correlated with the size of the sella turcica. However, the response to irradiation was independent of sella size. This is in contrast to surgery, where excellent results are obtained with small tumors, but distinctly inferior results are obtained with markedly enlarged pituitaries. In addition we studied the response of patients to treatment with bromergocryptine, which has been controversial. In our study, a minority of patients respond well, but the responsiveness of this group of patients may be masked in large group studies.

Several growth hormone related peptides have been studied in detail and have been closely related to their *in vivo* forms. At least one of these forms has an enhanced ratio of biological/immunological activity and could therefore be of significance in measurement of responses to therapy.

Generally patients with acromegaly have been included in larger groups of patients who have other prolactin-related problems, on the assumption that patients with acromegaly would be the same. We found that patients with acromegaly have a distinct pattern of plasma prolactin; 40% of the females and 25% of the males had elevated levels of circulating hormone. In patients who initially had elevated prolactin levels, irradiation produced a small fall over the next several years while those with normal prolactin levels showed a rise after irradiation. Furthermore, a correlation between plasma growth hormone and plasma prolactin was demonstrated both in the basal state, in patients followed over several years without treatment, as well as patients treated with irradiation, and in one smaller group of patients treated with surgery. Galactorrhea with acromegaly was also investigated; the galactorrhea might be due to an elevation in growth hormone without an elevation in plasma prolactin due to spillover effects at the level of the receptor.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 47001-01 DB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Phosphorylation of the Insulin Receptor.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Y. Zick	Guest Worker	DB NIADDK
OTHER:	J. Whittaker	International Fogerty Fellow	DB NIADDK
	S.I. Taylor	Medical Officer	DB NIADDK
	G. Grunberger	Clinical Associate	DB NIADDK
	P. Gorden	Clinical Director, NIADDK	DB NIADDK
	J. Roth	Chief, Diabetes Branch	DB NIADDK

COOPERATING UNITS (if any)

N/A

LAB/BRANCH
Diabetes Branch

SECTION
Receptor and Hormone Action

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.7	2.7	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Insulin stimulates phosphorylation of both α and β-subunits of its own receptor in a cell-free system using a solubilized, partially purified preparation of insulin receptors from rat liver membranes. The receptor subunits are isolated by specific immunoprecipitation with anti-receptor antibodies followed by SDS-gel electrophoresis. Under reduced conditions two bands, 95K and 135K, were selectively labeled which correspond exactly to the position of the α and β subunits of the insulin receptor. Phosphorylation of both subunits was stimulated 4-6 fold with pork insulin. Guinea pig insulin was about 2% as potent as pork insulin, whereas EGF, ACTH, and glucagon were ineffective. This process suggests that phosphorylation is an early step in insulin action.

SMITHSONIAN SCIEN. . INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 47003 - 06 DB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Insulin Receptors and Insulin Responsiveness in 3T3-L1 Fatty Fibroblasts

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	C.R. Kahn	Senior Surgeon	DB, NIADDK
Other:	K.L. Baird	Biologist	DB, NIADDK
	C. Grunfeld	Assist Prof of Med	Univ California
	C.-C. Wang	Guest Worker	DB, NIADDK
	M. Crettaz	Guest Worker	DB, NIADDK

COOPERATING UNITS (if any)

Department of Medicine, University of California, San Francisco, CA

LAB/BRANCH
Diabetes Branch

SECTION
Cellular and Molecular Physiology Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0		

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project has been terminated.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 47005-10 DB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Studies of Insulin Binding in Circulating Cells of Man.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. Gorden	Medical Director	DB, NIADDK
OTHERS:	R.F. Dons	Clinical Associate	DB, NIADDK
	G. Grunberger	Clinical Associate	DB, NIADDK
	J. Ryan	Bio Lab Tech	DB, NIADDK
	C.M. Hendricks	Bio Lab Tech	DB, NIADDK

COOPERATING UNITS (if any)

None

LAB/BRANCH
Diabetes Branch

SECTION
Clinical and Cellular Biology Section and Receptors and Hormone Action Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 3.2	PROFESSIONAL: 2.2	OTHER: 1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Numerous studies from this laboratory have investigated the interaction of insulin with circulating mononuclear cells. The present project involves studies in vivo insulin-dependent diabetes mellitus (IDDM) and glucocorticoid excess. We have attempted to characterize the insulin receptor of the human erythrocyte. Mononuclear cells and erythrocytes both have insulin receptors whose functions are altered by conditions of health and disease. Comparative studies of these two different cell types show that in some cases their insulin receptor status may be similar, but that the actual regulation of their receptor concentration may differ.

The effects of sulfonylurea drugs on erythrocytes and mononuclear cells were also studied. These drugs do not seem to produce their clinical effect by direct interaction with the insulin receptor.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 47007-07 DB

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Antibodies to receptors: Detection in disease states and use as probes to study receptor function and structure.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. Gorden	Clinical Director, NIADDK	DB NIADDK
OTHERS:	S.I. Taylor	Medical Officer	DB NIADDK
	L.H. Underhill	Chemist	DB NIADDK
	B. Samuels	Chemist	DB NIADDK
	R. Dons	Clinical Associate	DB NIADDK
	K. Baird	Biologist	DB NIADDK
	J. Roth	Chief, Diabetes Branch	DB NIADDK
	J. Ryan	Biological Lab Technician	DB NIADDK
	R.M. Pruss	Staff Fellow	LCS NIMH
	C. Lane	Clinical Associate	LIR NIAID
	A. Fauci	Chief, Lab of Immunoregulation	LIR NIAID
	G. Whalen	Biologist	LIR NIAID

COOPERATING UNITS (if any)

Laboratory of Clinical Sciences, National Institute of Mental Health; Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases

LAB/BRANCH

Diabetes Branch

SECTION

Clinical and Cellular Biology Section

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 4.2	PROFESSIONAL: 2.2	OTHER: 2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Autoantibodies to the insulin receptor occur in patients with insulin resistance as well as patients with hypoglycemia. We have carried out in vitro and in vivo studies of the effects of anti-receptor antibodies to mimic or antagonize insulin action. We are attempting to address the question of what determines the balance between the insulinomimetic and insulin antagonistic actions of anti-receptor antibodies.

We have obtained B-lymphocytes from the peripheral blood and bone marrow from patients with anti-receptor antibodies in an effort to establish hybridomas which would synthesize anti-receptor antibodies in vitro. These monoclonal antibodies would be extremely useful as probes of receptor structure.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 47010-10 DB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Patient Studies Using MSA (Multiplication Stimulating Activity) Radioreceptor Assay.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. Gorden	Clinical Director, NIADDK	DB NIADDK
OTHERS:	C.M. Hendricks	Medical Technologist	DB NIADDK
	R.E. Silverman	Medical Staff Fellow	DB NIADDK

COOPERATING UNITS (if any)

N/A

LAB/BRANCH
Diabetes Branch

SECTION
Clinical and Cellular Biology Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 2.2	PROFESSIONAL: 1.2	OTHER: 1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

MSA (Multiplication Stimulating Activity) is an insulin-like peptide which reacts with its own receptor and the insulin receptor. MSA is elevated in about 40% of patients with hypoglycemia and non-islet cell tumors. Patients with hemangiopericytoma have elevated circulating MSA levels. Cells from two hemangiopericytoma patients have been put into tissue culture. Analysis of proteins from both the cells and the conditioned media suggests production of both an insulin-like growth factor and a binding protein.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 47014 - 13 DB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Acromegaly and Growth Hormone

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	P. Gorden	Medical Director	DB, NIADDK
Other:	F. de Pablo	Visiting Fellow	DB, NIADDK
	N. Hizuka	Visiting Associate	DB, NIADDK
	J. Roth	Medical Director	DB, NIADDK

COOPERATING UNITS (if any)
None

LAB/BRANCH
Diabetes Branch

SECTION
Clinical and Cellular Biology Section and Receptors and Hormone Action Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 1.4	PROFESSIONAL: 1.4	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The standard therapy for growth hormone elevation in acromegaly has been conventional supervoltage irradiation. The data of human prolactin levels in acromegalics who received radiotherapy has been elevated at 2, 5, and 10 year follow-ups. The data suggest a correlation between prolactin and human growth hormone not only at the level of pituitary secretion but possibly also at the target cell. Patients treated with supervoltage irradiation were evaluated. The results showed that prior to surgery basal growth hormone levels were positivity correlated with estimate of tumor size in acromegalic patients, however, the effectiveness of radiotherapy with regard to percent reduction in growth hormone levels is largely independent of the degree of sella abnormality.

Bromocryptine as therapy for acromegalics was studied. The effectiveness of bromocryptine in lowering plasma hGH concentration remains ambiguous. Variant forms of hGH extracted from pituitary and obtained by genetic engineering techniques have been studied in radioreceptor- and radioimmunoassays.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AM 47016 - 05 DB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
The Structure of the Insulin Receptor Studied by Ionizing Radiation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	C.R. Kahn	Senior Surgeon	DB, NIADDK
Other:	J.T. Harmon	Guest Worker	DB, NIADDK
	S.E. Kempner	Senior Investigator	LPB, NIADDK

COOPERATING UNITS (if any)
Laboratory of Physical Biology, NIADDK

LAB/BRANCH
Diabetes Branch

SECTION
Cellular and Molecular Physiology Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER
☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project has been terminated.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 47018 - 05 DB

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Cellular Hormone-Like Peptides

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	J. Roth	Chief, Diabetes Branch	DB, NIADDK
OTHERS:	D. LeRoith	Visiting Scientist	DB, NIADDK
	M.A. Lesniak	Chemist	DB, NIADDK
	F. de Pablo	Visiting Fellow	DB, NIADDK
	E. Hernandez	Visiting Associate	DB, NIADDK
	E. Collier	Medical Staff Fellow	DB, NIADDK
	S.A. Hendricks	Guest Worker	DB, NIADDK
	C.-D. Agardh	Visiting Fellow	DB, NIADDK
	J. Shiloach	Visiting Scientist	LNE, NIADDK
	J. Rosenzweig	Assistant Professor	Case Western Reserve Univ.

COOPERATING UNITS (if any)

Laboratory of Nutrition and Endocrinology, NIADDK; Case Western Reserve Univ., Cleveland, Ohio.

LAB/BRANCH

Diabetes Branch

SECTION

Receptors and Hormone Action Section

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 5.8	PROFESSIONAL: 5.8	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

To further substantiate our earlier claim that unicellular organisms make vertebrate peptide hormones, we have extended our studies. Thus, we studied additional strains of tetrahymena pyriformis, a citiated protozoan as well as three new strains of E. coli. In each instance, we have found material very similar to insulin. These similarities include chromatography on ion exchange sephadex columns, reverse phase hydrophobic interactions and radioimmunoassay as well as bioassay. The production of insulin by both tetrahymena and E. coli during their growth cycles were studied; insulin can be recovered from extracts of the cells as well as from the media during the logarithmic growth phase.

In a preliminary fashion in extracts of tetrahymena, other hormones are found to be present: cholecystokinin, glucagon, arginine, vasotocin, and relaxin.

Further extension of this study shows that insulin is present in the chick embryo before the beta cell is recognizable which extends observations that insulin is native to organisms that lack pancreatic islets, including flies, worms, and microbes.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 47019 - 05 DB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Morphologic Studies of Ligand Binding to Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	P. Gorden	Medical Director	DB, NIADDK
Other:	J.-L. Carpentier	Chargé de Recherche	Univ of Geneva
	M.A. Lesniak	Chemist	DB, NIADDK
	N. Hizuka	Visiting Associate	DB, NIADDK
	L. Orci	Prof of Medicine	Univ of Geneva

COOPERATING UNITS (if any)
Institiute of Histology and Embryology, University of Geneva, Geneva, Switzerland

LAB/BRANCH
Diabetes Branch

SECTION
Clinical and Cellular Biology Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 2.3	PROFESSIONAL: 2.3	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

^{125}I-insulin binds to IM-9 cells (human) and hepatocytes (rat). By using quantitative EM autoradiography as a probe, it can be shown that labeled insulin is internalized by a process which appears to be time- and temperature-dependent. Studies indicate that the microvillus surface is involved in initial hormone binding and the receptors are lost from this surface in a time- and temperature-dependent manner. Other labeled proteins (low density lipoprotein, epidermal growth factor, human growth hormone, glucagon, and anti-insulin receptor antibody) appear to behave in the same fashion i.e. initial binding followed by internalization.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 47021-04 DB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Receptors and Hormone Action in Cultured Human Fibroblasts

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J.M. Podskalny	Biologist	DB NIADDK
OTHERS:	P. Gorden	Clinical Director, NIADDK	DB NIADDK
	S. Takeda	Visiting Associate	DB NIADDK

COOPERATING UNITS (if any)

N/A

LAB/BRANCH
Diabetes Branch

SECTION
Cellular and Molecular Physiology Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 2.2	PROFESSIONAL: 2.2	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Cultured human fibroblasts are being used to look for genetic receptor and post-receptor defects in insulin resistant patients. Insulin stimulated glucose uptake and α-amino-isobutyric acid (AIB) transport have been examined in cells from an infant with leprechaunism. Fibroblasts from this patient express low affinity insulin receptors. Both of the transport systems appear to function normally in the receptor-mutant cells. The binding of epidermal growth factor (EGF) is markedly reduced in the leprechaun cells. High media glucose levels can partially correct the abnormal insulin binding in the leprechaun cells but not the EGF binding. Other studies with EGF show that in normal fibroblasts, EGF will blunt insulin stimulated activation of glycogen synthase, glucose uptake, and AIB transport. Human growth hormone will also slightly inhibit glucose uptake in these cells, both basally and in the stimulated state.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 47022 - 03 DB

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)
Insulin Receptors in Syndromes of Extreme Insulin Resistance

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	S.I. Taylor	Medical Officer	DB, NIADDK
Other:	J.A. Hedo	Visiting Associate	DB, NIADDK
	A. McElduff	Guest Worker	DB, NIADDK
	J. Whittaker	Int. Fogarty Fellow	DB, NIADDK
	L.H. Underhill	Chemist	DB, NIADDK
	B. Samuels	Chemist	DB, NIADDK
	J. Roth	Medical Director	DB, NIADDK
	P. Gorden	Medical Director	DB, NIADDK
	E. Hernandez	Visiting Associate	DB, NIADDK
	L.M. Wagman	Surgeon	SB, NCI

COOPERATING UNITS (if any)
Surgery Branch, National Cancer Institute

LAB/BRANCH
Diabetes Branch

SECTION
Receptors and Hormone Action Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.5	4.5	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Using Epstein-Barr virus, we have established cell lines from peripheral blood of normal subjects and patients with extreme insulin resistance, i.e. lipoatrophic diabetes, leprechaunism, type A extreme insulin resistance, and Rabson-Mendenhall syndrome. Three classes of patients have been defined: 1) those with a decreased number of normal insulin receptors; 2) those with a normal number of qualitatively abnormal insulin receptors; and 3) those with a normal number of receptors without any detectable abnormality in insulin binding.

In all of the patients, we are carrying out direct studies of insulin receptor structure in an effort to determine whether there is a structural abnormality. With cells from the patients with a decrease in receptor number, we are studying rates of receptor turnover to ascertain whether the abnormality results from a decrease in the rate of synthesis or an increase in the rate of receptor degradation. In cells from one of the patients with extreme insulin resistance, we have identified a decrease in the sensitivity of insulin binding to alterations in pH and temperature. In addition, there appear to be defects in receptor cooperativity and the regulation of receptor affinity.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 47023 - 03 DB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Mechanism of Action of Insulin and Insulin-Like Growth Factors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	C.R. Kahn	Senior Surgeon	DB, NIADDK
Other:	G.L. King	Senior Assist Surgeon	DB, NIADDK

COOPERATING UNITS (if any)

NONE

LAB/BRANCH
Diabetes Branch

SECTION
Cellular and Molecular Physiology Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project has been terminated.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 47,024 - 03 DB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Biosynthetic Labeling of the Insulin Receptor

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	J.A. Hedo	Visiting Associate	DB, NIADDK
Others:	S. Cushman	Assoc. Chief	CMOS, NIADDK
	I. Simpson	Visiting Associate	CMOS, NIADDK
	C.-C. Wang	Visiting Fellow	DB, NIADDK
	C.R. Kahn	Research Director	Joslin Diabetes Center
	M. Kasuga	Research Fellow	Joslin Diabetes Center

COOPERATING UNITS (if any)
Cellular Metabolism and Obesity Section, NIADDK
Joslin Diabetes Research Center, Boston, Massachusetts

LAB/BRANCH
Diabetes Branch

SECTION
Cellular and Molecular Physiology Section; Receptors and Hormone Action Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The insulin receptor of cultured human lymphocytes has been characterized by biosynthetic labeling with radioactive amino acids (methionine and leucine) and sugars (fucose, galactose, glucosamine and mannose). In addition the subunits of the receptor have been labeled by cell surface methodology that reveal proteins (lactoperoxidase/Na^{125} I) or glycoproteins (galactose oxidase/NaB^3H_4, periodate/NaB^3H_4). The receptor has, as shown by all these methods, two major subunits of molecular weights 135,000 and 95,000 and a minor component of 200,000-210,000. The two major subunits are also revealed by affinity cross-linking with ^{125}I-insulin. The 200,000 component appears to be intracellular high mannose glycoprotein. The 210,000 component appears to be a fully glycosylated, but uncleaved, proreceptor by peptide mapping. In addition, further studies suggest that insulin induces the internalization of both major subunits of the receptor and the 95,000 subunit is a transmembrane protein while the 135,000 subunit is exposed only on the extracellular surface of the plasma membrane.

ANNUAL REPORT OF THE PEDIATRIC METABOLISM BRANCH

Clinical and laboratory investigations of cystic fibrosis (CF), one of the commonest serious inherited diseases, were continued in the fiscal year 1981-82.

I. GLYCOPROTEIN STUDIES

Oligosaccharide Structure of Human α_2-Macroglobulin in Normal Controls and Patients with CF:

CF is characterized by an abnormal physico-chemical behavior of mucous secretions leading to obstruction of organ passages and consequent sympotmatology (e.g.: chronic pulmonary disease, pancreatic deficiency, cirrhosis, pulmonary disease, intestinal obstruction).

For some time, it has been postulated that the basic defect in CF lies in the carbohydrate moieties of glycoproteins and mucopolysaccharides. Several studies have reported changes in the sugar composition of mixtures of glycoproteins or mucins. No study has systematically investigated the oligosaccharide structure of a purified glycoprotein from a patient with CF and compared the structure to that of the normal glycoprotein. A good candidate for this type of study is α_2-macroglobulin. α_2-Macroglobulin from patients with CF has altered binding to Con A, an indication of a change in the carbohydrate structure of the glycoprotein, and one group found that α_2-macroglobulin had lower levels of sialic acid than did the α_2-macroglobulin from normal volunteers. In addition, the oligosaccharide structure of normal human α_2-macroglobulin has not been determined previously.

Accordingly, α_2-macroglobulin was isolated from the plasma of seven patients with CF and seven normal individuals by a combination of previously published methods. Around 250-400 mg of glycoprotein was isolated from 350 ml of plasma from each subject. Sugar and methylation analyses of the intact glycoprotein from each individual showed little variation. The sialic acid content of the α_2-macroglobulin from the patients with CF was normal.

Additional studies were performed on the glycopeptides obtained by treatment with pronase. These glycoproteins could be separated into three fractions by Con A-Sepharose chromatography. The distribution of the glycoproteins from the normal samples into the three fractions was virtually identical. In contrast, the distribution of the glycoproteins from the CF samples varied considerably, ranging from a normal distribution to no similarities at all. This result suggests that the factors controlling the types of chains that are attached to α_2-macroglobulin may be faulty.

The detailed structural analysis of each Con A-Sepharose fraction from the normals and from patients with CF is nearly finished. When all the data are available the structure of the carbohydrate chains in normal human α_2-macroglobulins will be clarified.

As yet, no conclusions can be drawn as to differences between normal controls and patients with CF. This study includes sugar and methylation analysis before and after Smith degradation of the glycoprotein. Sequential digestions with glycosidases will help establish sequence and anomeric configurations. The information from these studies will define the oligosaccharide structures on each of the isolated glycopeptides and allow a definitive comparison of the carbohydrate chains from CF patients with those from normals (Mark E. Lowe, Ph.D., NIADDK).

II. THE FAT CELL AS A MODEL IN CF

Altered Sensitivity to Catecholamines in CF:

There are no animal models for CF and the cultured skin fibroblast from CF patients has proved unreliable and unsatisfactory in this disorder. On the other hand, adipose tissue appears to be a very good model as it can be easily obtained by biopsy from patients and in answer to a number of challenges the fat cell secreates glycerol which can be titrated and therefore gives a quantitative answer.

Earlier studies in this Branch (Davis et al: NEJM 302:1453, 1980) had shown that CF patients were hyporesponsive to α-adrenergic and cholinergic drugs, but resistant to β-adrenergic drugs as determined by pupillary responses and cardiovascular changes. These findings were consistent with our observation (Davis et al: Pediat. Res. 12:703, 1978) that leukocytes from CF patients produce less cAMP on stimulation in vitro with catecholamines than do cells of control subjects. Accordingly, these studies were extended by determining the sensitivity to catecholamines in a physiologically responsive system, the mature fat cell.

Adipose tissue was obtained by needle biopsy from the gluteal area of CF patients and appropriate controls. Adipocytes were prepared by a micro-modification of Rodbell's procedure and were incubated at 37°C in 0.5 ml of Krebs-Ringer Buffer with 2% bovine serum albumin and 5 mM glucose for 2 hours.

From 3 to 20 X 10^3 fat cells were present in each incubation vessel. The release of glycerol (lipolysis of fat cell triacylglycerol) was stimulated by addition to the incubated medium of isoproterenol (10^{-8}-10^{-5}M), Theophylline (10^{-4}M), Dibutyryl cyclic AMP (10^{-3}M) or a combination of these.

Isoproterenol binds to β-adrenergic receptors in the cell plasma membrane and activates the receptor-adenylate cyclase complex, resulting in activation of hormone-sensitive lipase and release of glycerol and fatty acids from the fat cell. Theophylline, which inhibits phospho-diesterase, and Dibutyryl cyclic AMP, activates the lipase in the same manner, but enters the system below receptor level. Glycerol release was expressed either in terms of DNA (cell number) or triacylglycerol (cell mass) counts of the adipocytes.

Basal lipolysis and the lipolytic response to isoproterenol was virtually identical for adipocytes of CF patients and controls. Effects of theophylline and dibutyryl cyclic AMP on the cell were also the same in the two groups. No evidence of impaired physiological response of CF adipocytes to β-adrenergic agents was detected in this study.

We concluded, therefore, that systemic resistance to stimulation by sympathomimetic amines does not occur in CF and any genetic defect in receptor-adenylate-cyclase complex is organ or cell specific.

The isolated adipocytes will be employed in other series of studies to determine whether CF alters the ability of the cells to take up cations (Ca, K, Na, etc.) and whether the responses of this system to various hormones (insulin, etc.) are normal (Sidney S. Chernick, Ph.D., Milica Spasovska-Chernick, M.D., NIADDK).

III. CLINICAL STUDIES IN CF

Studies of Intestinal Malabsorption in CF:

Lingual lipase is a very interesting enzyme which has been overlooked until recent times. It is very important in the intragastric digestion of fat in newborns and infants and presumably in other conditions in which pancreatic lipase is not available, as in most patients with CF. Studies by our group show that gastric lipolysis in CF did, in fact, show that lingual lipase is not only present, but even increased in CF, perhaps as a compensatory mechanism. Lingual lipase is resistant to acid and its activity is independent of bile acids, ideal characteristics in CF, where pH and bile acid concentrations are known to be low. In normal subjects lingual lipase would be inhibited in the duodenum by the presence of bile acids and inactivated by the higher luminal pH. However, in CF patients our studies show that the pH of the duodenal environment is favorable for continued activity and bile acid concentration is usually reduced compared to normal levels. Lingual lipase supplements should be considered for the treatment of the malabsorption aspects of CF and would probably be much more effective than the supplements of pancreatic enzymes traditionally used in this disease.

In a series of studies the postprandial duodenal secretions in the CF patients with exocrine pancreatic insufficiency were shown to have a pH of 4.80 ± 0.3 (mean ± SD) compared with a pH of 7.0 ± 2 in normal subjects. Accordingly, the efficacy of delivering exogenous pancreatic enzymes into the duodenum of CF patients was evaluated comparing the new pH sensitive enteric coated pancreatic microspheres with the previously available non-enteric coated pancreatic enzyme supplements. A duodenal perfusion technique was used with non-absorbable gastric (PEG) and duodenal (^{14}C-PEG) markers, and a standard meal (Lundh meal) was administered orally with either pancreas(enteric coated) or cotazyme (non-enteric coated) commercial preparations. The dosage of enzyme preparation given was adjusted so that 32,000 units of lipase activity were administered. We found that the cumulative post-prandial delivery of enzymes to the ligament of Treitz was significantly lower with the enteric coated preparations than with the non-enteric form. Since the pH sensitive enteric coating is formulated to allow optimal release of the enzyme at a pH of 6.5, these results can be explained by the suboptimal pH in the small intestinal environment.

The knowledge gained through these studies will help provide alternative therapeutic manipulation to improve the maldigestion and malabsorption present in approximately 90% of patients with CF.

Cimetidine, an H_2-receptor antagonist, decreases gastric acid output and thereby limits gastric inactivation of supplemental pancreatic enzymes. Decreased gastric acid secretion would also be expected to increase duodenal pH and allow better release of the supplemental enzymes from the pure enteric coated preparations. Indeed, improved coefficients of fat and nitrogen absorption have been observed in all patients evaluated. Additionally, in our studies we have not observed any of the most frequently reported side effects.

Bacteriologic Studies:

A series of bacteriologic studies were undertaken to define the respiratory and gastro-intestinal flora of patients with CF. We reviewed 874 cultures from 71 patients followed at the NIH from January 1977 through July 1981. There were 64 isolates of Haemophilus species; H. influenzae accounted for 54 isolates (85%), all were non-typable. Seven isolates of H. influenzae and one isolate of H. parainfluenzae were resistant to ampicillin due to beta-lactamase production. Two isolates of H. influenzae were also resistant to chloramphenicol. Ampicillin resistant H. influenzae were isolated from both clinically well and clinically ill patients. Other studies include the incidence of Clostridium difficile and of Giardia lamblia in the stools of CF patients as they both may exacerbate gastro-intestinal symptoms.

Other Complications of CF:

Appendiceal abcesses are a difficult problem in diagnosis and treatment in CF because they are often masked by the antibiotic therapy patients are receiving for their pulmonary disease. As no study of this problem is available in the literature, a review of the five cases seen at the NIH is being undertaken to establish guidelines for the management of CF patients with this condition.

There has been a report of *ovarian cysts* in CF patients. Routine pelvic ultrasonography is being performed. It appears that there is a very significant increase in this complication in CF patients (Paul A. di Sant'Agnese, M.D., Milica Spasovska-Chernick, M.D., Vincent P. McCarthy, M.D., Van S. Hubbard, M.D., Ph.D., NIADDK).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 49001-09 PMB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Clinical Studies in Cystic Fibrosis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Paul A. di Sant'Agnese, M.D. Chief, PMB NIADDK

OTHER: Van S. Hubbard, M.D., Ph.D. Senior Surgeon PMB NIADDK
Milica S. Chernick, M.D. Staff Fellow PMB NIADDK
Mark E. Lowe, Ph.D. Staff Fellow PMB NIADDK
Robert Fink, M.D. Guest Worker PMB NIADDK
Vincent P. McCarthy, M.D. Staff Fellow PMB NIADDK

COOPERATING UNITS (if any)
Sudhir Dutta, M.D. University of Maryland
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Robert Wolf, D.D.S. NIDR

LAB/BRANCH
Pediatric Metabolism Branch

SECTION

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6	4	2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Cystic fibrosis is a generalized exocrinopathy in which the basic defect is still unknown. Studies have been pursued to further define abnormalities observed in these patients. Investigations demonstrating the presence of lingual lipase activity in the duodenum and the more acidic duodenal pH in the CF patients and its effect on the efficacy of exogenous pancreatic enzymes has stimulated the development of better therapeutic intervention of the digestive and nutritional problems observed in most CF patients.

Thorough characterization of our CF population by both standard and investigational diagnostic criteria (ultrasonography, microbiological cultures, isoamylase) has allowed us to determine the incidence of ovarian cysts, unusual microbiological flora, and follow the progression of the pancreatic involvement in relationship to other characteristics of the disease; i.e.: pulmonary and nutritional status, and severity of infection. These correlations will aid in further clinical evaluations as well as in improving treatment.

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U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 49004-03 PMB

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Fatty Acids and Membrane Function in Cystic Fibrosis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Van S. Hubbard, M.D., Ph.D. Senior Surgeon PMB NIADDK

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3	2	1

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☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Altered fatty acid composition (decreased linoleic acid and increased palmitoleic and oleic acids) is found in blood and tissue lipids of patients with cystic fibrosis. Although these alterations are related to the malabsorption and maldigestion associated with pancreatic insufficiency in the CF patient, recent investigations suggest that the lower caloric intake of the CF patient relative to their caloric requirements compared to normal individuals may lead to increased utilization of absorbed essential fatty acids (i.e.: linoleic acid). Such increased metabolism of linoleic acid would explain the altered fatty acid composition observed.

The decreased linoleic acid content as observed in most cystic fibrosis patients is associated with decreased unsaturation in tissue phospholipids and membranes. This characteristic may relate to changes in membrane function as well as altering prostaglandin synthesis. Current work is aimed at delineating the role of abnormal fatty acid composition of blood and membrane lipids as a cause of alterations in the recognition or interaction of the beta-receptors in CF tissues and alteration in the availability of arachidonic acid for the biosynthesis of prostaglandins.

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PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Follow-up of Hypochloremic Infant Formula Feedings

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Van S. Hubbard, M.D., Ph.D. Senior Surgeon PMB NIADDK

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☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In the summer of 1979, a new clinical syndrome in infants less then 10 months of age characterized by poor appetite, failure to thrive, hypochloremia and varying degrees of metabolic alkalosis was described in association with the feeding of a chloride deficient formula, Neo-Mull-Soy. Twenty-two of the approximately 148 infants, reported to and confirmed by the Center for Disease Control as having this syndrome have been studied since long-term effects of chloride deficient feedings in humans are not known. Evaluation at an average of 24-28 mo. of age demonstrated normal routine blood chemistries and a normal metabolic response to alterations of sodium intake. Other underlying known metabolic disorders have not been identified. Repeat evaluations with emphasis on psychometric testing are currently being performed.

At the present time there is no common characteristic or symptom observed in these infants as a result of the chloride deficient feeding.

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PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Oligosaccharide Structures on Human α_2-Macroglobulin

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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COOPERATING UNITS (if any)

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LAB/BRANCH

Pediatric Metabolism Branch

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INSTITUTE AND LOCATION

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☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

α_2-Macroglobulin was isolated from the plasma of normal individuals and patients with cystic fibrosis. An analysis of the carbohydrate structures on these homogenous preparations is ongoing. The preliminary results indicate that the α_2-macroglobulin from normal and cystic fibrosis patients have similar sugar compositions, but that the oligosaccharide chains may have different sequences or linkages.

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PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

The Fat Cell as a Model in Cystic Fibrosis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Milica Spasovska-Chernick, M.D. Staff Fellow PMB NIADDK

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Sidney S. Chernick, Ph.D. LCDB NIADDK

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2	1	1

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Adipose tissue was obtained by needle biopsy from cystic fibrosis (CF) patients and controls. Adipocytes were incubated in Krebs-Ringer Buffer with 2% bovine serum albumin and 5 mM glucose for 2 hrs. The effects of Isoproterenol, Theophilline, and Dibutyryl cyclic AMP on hormone sensitive lipase were measured by glycerol release.

Basal lypolysis and lipolytic response to Isoproterenol were virtually identical for adipocytes of CF patients and controls. Effects of Theophilline and Dibutyryl cyclic AMP on the cell were also the same in the two groups. No evidence of impaired physiologic response of CF adipocytes to β-adrenergic agents was detected in this study.

ANNUAL REPORT SUMMARY

CLINICAL HEMATOLOGY BRANCH

I. Study of Immunology of Blood Cell Deficiencies

A. The Non-specific Nature of Platelet-associated IgG.

The increased amounts of immunoglobulin-IgG reported by many investigators to be on platelets of patients with ITP is generally assumed to be a measure of specific antiplatelet antibody responsible for the disease. However, our own observations raised doubts that this conclusion was correct. *In vivo* studies indicated that platelets coated with known antiplatelet antibodies in amounts equivalent to usual platelet-associated IgG (PAIgG) levels in ITP, were destroyed so rapidly that no platelets circulated. When platelets rose in ITP after steroid treatment or splenectomy, PAIgG became normal within several days to a week. Even if this type of treatment caused immediate cessation of antibody production, which is unlikely, the apparent disappearance of antibody was too rapid to be accounted for by antibody decay; and there is no evidence that steroid treatment, and certainly none that splenectomy, inhibits antibody attachment to platelets. Also, PAIgG elevations occurred in apparent non-immune disorders associated with platelet destruction; particularly noteworthy was the Wiskott-Aldrich syndrome, in which fragmentation of defective platelets may be involved in the thrombocytopenia. Moreover, results of indirect tests for serum antibody in ITP did not correlate with those of direct PAIgG assays. For example, values for PAIgG obtained with the indirect tests were elevated even when results of direct tests were normal; the direct PAIgG test on platelets of mothers whose infants had neonatal ITP could be normal; and sera from 3 ITP patients that had been used in early experiments to induce thrombocytopenia gave negative indirect PAIgG tests.

We first established by elution experiments that platelets have high affinity for retained normal plasma IgG; and that most specific platelet isoantibodies and drug antibodies have relatively low affinity. Specific antiplatelet antibodies, with notable exceptions, did not coat platelets sufficiently to be detectable in the face of the large amounts of nonspecific IgG normally present on platelets. Next it was found that elevated PAIgG in ITP was an attribute of the platelet rather than antibody per se. In determining this we first ruled out the possibility that the abnormality was due to platelet age by studying platelets of different average ages in dogs and man during recovery from intense plateletpheresis. We further ruled out the possibility that activation of platelets by agonists such as thrombin antibody, or ADP might increase the adsorptive capacity of platelets for nonspecific IgG. We then evaluated the possibility that platelet alteration in ITP might lead to elevated PAIgG by virtue of increasing the amount of membrane per platelet. To test this we used a platelet membrane marker, anti-Pl^{El}, which reacts with essentially all normal human platelets in a quantitatively similar fashion. Platelets from ITP patients with elevated PAIgG had 2 to 3 times more membrane marker per platelet than did platelets from normal individuals or from ITP patients with normal PAIgG.

To see what relationship the apparent increase in membrane bore to platelet size, platelets from these same ITP patients were sized electronically. Of great interest was the finding that those patients with elevated PAIgG had an unusual sizing pattern that included particles too small to be considered platelets. Further, the higher the PAIgG the greater the proportion of small particles and the highest PAIgG values were associated with a predominance of small particles. On electromicroscopy the small particles were found to consist primarily of platelet fragments, and platelet membranes devoid of cytoplasm. The relatively large surface area of these particles and their cytoplasmic deficiency accounted for increased levels of PAIgG based on platelet protein as well as on platelet number. Thus, platelet membrane debris, not antibody, determines the level of PAIgG. This finding accounts for all of the above mentioned paradoxical observations with respect to PAIgG. It further may provide insight into the nature of the platelet-destruction process and provides a basis for evaluating the cause of thrombocytopenic states without recourse to platelet survival studies, in addition to elucidating the dynamics of platelet interaction with normal and anti-platelet immunoglobulins.

B. PAIgG and Platelet-turnover in Systemic Lupus Erythromatosis (SLE).

The concept that most patients with SLE have shortened platelet survival owing to immunologic destruction is entrenched in the literature on the basis of relatively few studies involving platelet survival techniques, antibody assays and cell-sizing procedures. Supposedly in SLE survival is shortened, platelets contain elevated PAIgG, and platelet size is increased. None of the techniques that have been used is sufficiently accurate by current standards to document the conclusions concerning platelet turnover in SLE. Of 60 cases of SLE that we have studied only 20% had elevated PAIgG compared to over 65% reported by others. Of 6 patients in whom platelet survival tests were performed by the ^{51}Cr-label technique, none had shortened survival compared to the invariable shortened survival reported by others; and electronic sizing did not show large platelets compared to the reported sizing by micrometer. Furthermore, there was no EM evidence that SLE platelets with high PAIgG were fragmented like ITP platelets. Thus SLE platelets in 20% of patients appear to have an immunoglobulin coating that does not lead to excessive platelet destruction. Future studies will be directed at determining the nature of the immunoglobulin material on SLE platelets, whether it represents immune complexes, and, if so, the specificity of the antigen and antibody. Cases are currently under review to determine whether increased PAIgG in SLE has prognostic significance; non as yet is obvious.

C. Technique for Assaying Cell-bound Immunoglobulins.

A new assay has been developed for the measurement of cell membrane associated IgG, particularly that of platelets. The procedure involves reproducible acid elution of IgG from cell membranes followed by a solid phase immunoassay. The immunoassay involves linking specific anti-immunoglobulins to poly-lysine coated microtiter plates with gluteraldehyde followed by exposure of plates to neutralized acid eluates of cell membranes, then addition of a third layer of specific anti-immunoglobulin that is radiolabeled. This permits rapid measurement of numerous samples and exquisite sensitivity and specificity. Much controversy exists in the literature regarding the true amount of platelet-associated IgG (PAIgG) and its specificity, with some workers reporting values which are up to two orders of magnitude apart. Indications are that our new procedure provides an unambiguous measure of PAIgG.

D. A Technique for Evaluating Affinity of Interaction between Platelets and Immunoglobulins

To study the dynamics of platelet-immunoglobulin reactions a procedure was developed utilizing platelets chemically immobilized on resin beads. A chromatographic column filled with these immobilized platelets was used to characterize the binding of immunoglobulin from normal sera and sera from ITP patients. These studies clearly differentiated two degrees of affinity of normal immunoglobulin binding that nevertheless are competitive. The data may be interpreted as indicative that two binding sites are on the same membrane domain and close enough to compete with each other, perhaps by simple steric interactions. Alternately, there may be two types of IgG in the sera with varying affinities. When ITP patient sera were studied there was a curious decrease in binding of both classes. This is a surprising result in that ITP is generally thought to be an immune disease expected to be associated with increased binding. We are evaluating the possibility that there may actually be a low-affinity ITP antibody that interferes with binding of higher affinity normal immunoglobulins that predominate on platelet surfaces after washing procedures used in assays. Chromatographic procedures are currently being modified in attempts to detect the obscure ITP factor.

E. Identification of Isoantibody Receptors

This laboratory has accumulated a number of patients' sera which contain antiplatelet antibodies. These sera are being studied to characterize the specific platelet antigens against which they are directed. A Southern blotting procedure is used to transfer proteins from a SDS-polyacrylamide gel separation of platelet membrane proteins. These immobilized proteins are then reacted with the sera followed by an iodinated second antibody to visualize the reactive bands. Present data suggest that there is an unusual multiplicity of binding sites for normal immunoglobulin and additional single sites for specific antibodies. These findings are consistent with our studies showing large amounts of "normal sera" immunoglobulin binding to platelets at multiple binding sites.

F. Neuropeptide Studies in Adrenal Medullary Cells.

Primary cultures of adrenal medullary cells synthesize and secrete at least two neuropeptides. In collaboration with Dr. Lee Eiden of the Neuroendocrine Branch of NIMH we have identified the presence and release of vasoactive intestinal polypeptide in these cultures. The expression of this peptide, unlike the enkephalins (which have been shown by others and us to be present), appears to be induced during culture since VIP increases several fold after four days' culture. This is presently the only known culture system which contains VIP and will be quite useful to study the physiology and biochemistry of VIP. In collaboration with Dr. Eiden who has developed a complementary DNA for the proenkephalin mRNA, we plan to characterize the synthesis and turnover of mRNA in adrenal medullary cell cultures and changes in these parameters in response to various pharmacological manipulations.

G. Release of Arachidonic Acid from Adrenal Chromaffin Cell Cultures During Secretion of Epinephrine.

Membrane-associated biochemical changes during receptor mediated secretion are of fundamental importance to understanding events related to neurotransmitter secretion. This study shows that a transient release of arachidonic acid (AA) accompanies secretion of epinephrine from primary cultures of bovine adrenal chromaffin cells. Cultures were grown for 5 to 6 days, then labelled for 3 hours with ^{3}H-AA, washed and incubated for another hour. The cells were then stimulated with nicotine, acetyl choline or veratridine. Lipophilic constituents were extracted from the released media with ethyl acetate and chromatographed on TLC. The increase in radioactivity was found in a peak which cochromatographed with authentic AA. The increased release of ^{3}H-AA was complete within 5 min, with no further increase up to 30 min. The effects of nicotine, acetylcholine and veratridine were dose dependent with ED_{50}'s and between 5 and 25 μM. These results suggest that the activation of a phospholipase A and the liberation of arachidonic acid from phospholipids may be a step in the sequence of events which leads to secretion of neurotransmitters.

H. Antibodies to Neuronal Tissue in Sera of Patients with Dysautonomia.

When sera from a group of patients with idiopathic autonomic neuropathies were screened by radioimmunoassay for antibodies against cultured bovine adrenal medullary cells, 20% of the patient group had significantly higher binding than a comparable control group. Adrenal medullary cells were used as a source of neuronal antigens; but the same group of patients that had high IgG binding to chromaffin cells also had high binding to bovine platelets and rat hepatocytes, indicating that the antibodies are not neuronal specific. Moreover, different sera from patients with high binding reacted with different individual bands on trans-blot preparations of total chromaffin cell membrane components. Whether the antibodies in sera of patients with dysautonomia are cause or effect of the disease remains to be determined. In this respect studies are in progress to assess effects of these antibodies on cellular function such as secretion, desensitization and cell survival. Patients with diabetes, with and without autonomic disorders, will be studied to further evaluate the significance of anti-chromaffin cell antibodies. These antibodies also provide an opportunity to define chromaffin cell antigens, and possibly agonist receptors which would lead to more basic understanding of neurophysiologic function.

II. Study of Blood Coagulation and Diseases of Hemorrhage and Thrombosis

A. The Effects of Anion Transport-blocking Agents and Alterations of Osmotic Strength on the Interaction Between Platelets and Thrombin.

The ability of anion transport blocking agents and of elevated osmotic strength to inhibit exocytotic processes following stinulation of various cells or chromaffin granules by a variety of secretory agents led to the concept that the mechanism of exocytosis is a chemiosmotic process. The chemiosmotic theory suggests that the biochemical events in cellular secretion proceed to the point at which secretory granule fusion with plasma membrane occurs, then ionic transport and consequent elevated osmotic pressure leads to oncotic rupture in the final stage of exocytosis. Platelet secretion is suppressed by anion transport blocking agents and elevated osmotic strength in all respects similar to that of other secretory cells that have been studied. We found in our previous studies concerning the metabolic effects of anion transport blockers and high osmotic strength on platelets that, contrary to this theory, the biochemical responses to various stimuli were inhibited proportionately to inhibition of the platelet secretion. Moreover, anion transport blockers and high osmotic strength suppressed malondialdehyde formation which is the earliest metabolic response of platelets to stimulation. Furthermore, although anion transport blockers and high osmotic strength inhibited platelet release caused by thrombin, these inhibitors had no effect on release initiated by other stimuli such as ADP or A23187. These various observations are inconsistent with concepts of chemiosmotic regulation. Because the platelet receptor for thrombin may be platelet-associated fibrinogen we studied the effect of anion transport blockers and high osmotic strength on the conversion of fibrinogen to fibrin by thrombin. This reaction was inhibited to a degree comparable to the degree of inhibition of platelet secretion by similar concentrations of anion transport blockers and levels of increased ionic strength. We next determined that inhibition of platelet secretion was associated with a proportional suppression of thrombin binding to platelets. These observations provide evidence that the platelet thrombin receptor may be fibrinogen or a similarly reactive protein. Anion-channel blockers that combine with the thrombin receptor will be helpful in the isolation and characterization of this substance. Since it is clear that anion transport blockers and increased osmotic strength do not involve inhibition of a chemiosmotic step in platelet exocytosis as previously supposed, other cells that have been studied from this point of view should be re-evaluated to determine whether interference with agonist attachment to receptor accounts for the inhibitory action.

III. Mechanisms of Hormone and Transmitter Secretion

A. *In Vitro* Reconstitution of Chromaffin Granule-Cytoskeleton Interactions: Calcium Dependent F-actin Cross-linking by Purified Chromaffin Granule Membranes

The secretory vesicles of the adrenal medulla (chromaffin granules) provide a useful model system for biochemical investigation of the proposal that actin plays a role in exocytosis. We have made use of a novel application of falling ball viscometry to measure actin binding by membranes (Fowler *et al.*, and Luna *et al.*, J. Cell Biol., in press). Purified chromaffin granule membranes cause large increases in viscosity and gelation of purified rabbit skeletal muscle F-actin under conditions which may approximate the resting intracellular environment (pH 6.8, 20 mM KCl, 1 mM $MgCl_2$,$[Ca^{++}]_{free}$ ca. 10^{-8}M, 30°C). These increases in viscosity reflect actin binding to the membrane since F-actin is, in fact, observed to cosediment with chromaffin granule membranes under conditions which do not pellet F-actin alone. The actin cross-linking activity of the membranes under conditions which do not pellet F-actin alone. The actin cross-linking activity of the membranes is thermolabile (100°C, 2 min) and trypsin sensitive, suggesting that F-actin may interact with a protein component in the chromaffin granule membrane.

The increases in viscosity of F-actin induced by the membranes are somewhat enhanced by inclusion of 1-2 mM $MgCl_2$, and are greatly diminished by increasing KCl concentration, or pH over the pH range from pH 6.0 to 8.0. Increasing the free calcium ion concentration from ca. 10^{-8}M to ca. 10^{-6}M at pH 6.8 results in a 50-90% drop in the increases in viscosity induced by the membranes over the viscosity of F-actin. Regulation of the interaction of F-actin with chromaffin granule membranes by parameters (pH,$[Ca^{++}]_{free}$ which are thought to regulate secretion in vivo suggests that actin may indeed play a role in exocytosis.

B. A Cell-free Model for Compound Exocytosis: Aggregation and Fusion of Chromaffin Granules in the Presence of Calcium, Synexin and *cis*-Unsaturated

medulla that causes the calcium dependent aggregation of isolated chromaffin granules (J. Biol. Chem. 253:2858-2866 and 254:553-558). When chromaffin granules that have been aggregated by synexin are exposed at 37°C to *cis*-unsaturated fatty acids such as arachidonic acid (20:4) or oleic acid (18:1), they fuse together to form large, membrane-bound vesicles that swell to enclose the maximum volume. Only limited fusion can be induced by isomers of oleic acid in which the *cis* double bond is displaced towards or away from the head group, and no fusion is induced by *trans*-unsaturated or saturated fatty acids, detergents or lysolecithin. During fusion the soluble core proteins of the granules are retained although some of the epinephrine escapes. The swelling of the vesicles can be inhibited by increasing the osmotic strength of the medium, and we are investigating the role of osmotic forces in the fusion event.

The regulators of this *in vitro* fusion - calcium, synexin and *cis*-unsaturated fatty acids - may be present in the cytoplasm of the chromaffin cell when it is stimulated to release epinephrine and granule proteins by exocytosis. Therefore, this fusion event may also occur between chromaffin granules undergoing compound exocytosis.

C. Chemiosmotic Lysis of Chromaffin Granules In Vitro as a Model for Osmotic Events in Exocytosis in Vivo

Studies on several model systems of membrane fusion have suggested that the driving forces for fusion during exocytosis may include a gradient of osmotic strength across the site of plasma membrane-vesicle membrane interaction. Thus, regulation of the osmotic properties of intracellular compartments such as the secretory vesicle may be of importance during exocytosis. We have approached this potential regulatory mechanism by examining the molecular features of osmotic control in adrenal medullary chromaffin granules (CG). osmotic medium containing both MgATP and permeant anions such as chloride. This was due to an increase in CG osmotic content and subsequent water entry. Regulation of CG osmotic content by MgATP and anions reflected the involvement of several CG membrane components including a proton pumping ATPase and a selective anion transport site. Their concurrent actions resulted in electroneutral, coupled transport of protons and anions into the CG thereby elevating the CG osmotic content. This mechanism was elucidated by measuring the effects of osmotic strength, anions, MgATP and inhibitors of anion transport on CG lysis, transmembrane electrical and pH gradients, anion transport and ATPase activity in CG. The possible role of such chemiosmotic CG properties in exocytosis was supported by studies of secretion from isolated adrenal medullary chromaffin cells. Exocytotic secretion of catecholamines from these cells was dependent upon extracellular permeant anions, and was suppressed by elevated external osmotic strength and by the addition of proton ionophores (e.g. FCCP) or of some anion transport inhibitors.

D. Glucocorticoid Inhibition of (R) [C^{14}] Ascorbic Acid Uptake in Cultured Bovine Chromaffin Cells

ACTH administration causes abrupt release of ascorbic acid and glucocorti-system, supply medullary cells with cortical blood containing much higher concentrations of ascorbic acid and particularly glucocorticoids than found in peripheral blood. Dual secretion of ascorbic acid and glucocorticoids in this portal system could well have profound effects on adrenal physiology, but they have not been explored. We anticipated that glucocorticoids might influence ascorbic acid transport by medullary cells, and we studied the uptake of (R) [C^{14}] ascorbic acid into 1 day old cultured bovine chromaffin cells, looking for a possible effect of glucocorticoids on the uptake process. We found that uptake of ascorbic acid into cells was linear for at least 1 hour before it began to plateau, and that the Km was approximately 10^{-3} μM, well within the physiological range. The ascorbic acid uptake process was inhibited by 0°C or by dinitrophenol plus iodoacetate, and was dependent on cell number. Different glucocorticoids were then tested for ability to modify ascorbic acid transport. Dexamethasone, hydrocortisone, and cortisone were good inhibitors while hydrocortisone 21-sodium hemisuccinate was totally inactive. The inhibition was dose dependent, with cortisone exerting the greatest inhibition of the steroids tested. The K_i for hydrocortisone was approximately 50 μM, a value well within the range of corticosteroid concentration in the portal system after a significant stress. We take these data to indicate that both ascorbic acid release and re-uptake may be important as integral and regulated aspects of the adrenal stress response.

E. Cranule Fusion and Fission (Discharge) in Insulin Release from Islets of Langerhans are Biochemically Dissociable Events

Insulin release involves contact between the secretory vesicle and the plasma membrane (fusion), followed by discharge of insulin by lysis of the membranes at the contact site (fission). As an index of fusion, we followed the recruitment of granulebound somatostatin receptors to the islet surface, an event which accompanies secretion vesicle migration and insulin secretion. Granule fission was monitored by measuring insulin release. Substitution of NaCl by the impermeant salt Naisethionate led to an 80% decrease in glucosestimulated insulin release, with no inhibition f somatostatin receptor recruitment. By contrast, the phenothiazine drugs trifluoperazine and promethazine, believed to inhibit calciumsensitive proteins involved in stimulussecretion coupling, blocked insulin release and somatostatin receptor recruitment in parallel, both drugs having similar potencies (50% inhibition at 110 μM). IBMX (isobutylmethylxanthine) appears to stimulate specifically granule fission since IBMX-induced insulin release occurs without an increase in somatostatin receptor recruitment. Naisethionate which inhibits granule lysis blocked IBMX-stimulated insulin release.

Conclusions: 1. Granule fusion and the subsequent fission leading to hormone discharge, are distincet and separable events in exocytosis. 2. Calcium sensitive events in release, as inhibited by phenothiazines, probably occur at steps prior to fusion of the membranes. 3. Some pharmacologic and physiologic modulators of hormone release may act predominantly at the final exocytotic step of fission (e.g. isobutylmethylxanthine).

F. Calcium Specific Binding of Synexin to Chromaffin Cell Plasma Membrane

Synexin, a soluble calcium binding protein isolated from bovine adrenal medullary tissue, binds to chromaffin granules and causes them to aggregate in a calcium dependent manner (Creutz *et al*, *J. Biol. Chem.* *253*, 2858, 1978 and *254*, 555, 1979). It has been proposed that synexin may initiate membrane fusion during exocytosis by causing chromaffin granules to become closely juxtaposed to found to bind to plasma membrane coated beads and chromaffin granule membrane coated beads but not to uncoated beads nor to erythrocyte membrane coated beads, indicating membrane specificity. The analysis of enzyme markers showed that the binding of synexin to plasma membrane coated beads cannot be explained by contamination of the beads with granule membrane. The binding was calcium dependent (K_d 200 μM); magnesium, strontium and barium were ineffective substitutes. Synexin binding to beads was saturable and about half of the bound synexin could be recovered by incubation in the presence of EGTA. Synexin binding to membrane coated beads was also demonstrated by SDS polyacrylamide gel electrophoresis with comarable results. We conclude that synexin can bind specifically to the inner aspect of the plasma membrane of chromaffin cells and may therefore play a role in attaching chromaffin granules to the plasma membrane prior to exocytosis.

G. Hydrogen and Phosphorus Nuclear Magnetic Resonance Studies of Chromaffin Granules and Isolated Granule Components

We have studied the pH dependence of the chemical shifts of ^1H and ^{31}P resonances from the intragranular ATP to probe the structure of the granule matrix. The titration patterns of the resonances from the phosphate and the H_2 adenine proton of intragranular ATP are unusual in that they depart from the Henderson-Hasselbalch form normally observed for NMR titrations. A variety of model systems designed to duplicate the intragranular matrix have also been investigated to attempt to explain the anomalous titration patterns. Significant changes are observed in the titration patterns of the phosphate and the H_2 adenine proton of ATP in our model systems. These observations indicate that interactions exist between ATP, adrenaline and the granule matrix and that these elements are essential for reinstituting the environment around the ATP *in vitro*.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 51,000-24 CHB

PERIOD COVERED

October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Study of Immunology of Blood Cell Deficiencies

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	N. Raphael Shulman	Chief	CHB	NIADDK
Other:	Adair Hotchkiss	Staff Fellow	CHB	NIADDK
	Cindy Leissinger	Clinical Associate	CHB	NIADDK
	Carol Kautz	Biologist	CHB	NIADDK

COOPERATING UNITS (if any)

Arthritis and Rheumatism Branch, NIADDK
Neuroendocrine Branch, NIMH

LAB/BRANCH
Clinical Hematology Branch

SECTION

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.75	2.0	.75

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The immunologic disorders, idiopathic thrombocytopenic purpura (ITP), lupus erythromatosis, neonatal-, post-transfusion-, and drug-purpura have been further studied with respect to determining pathophysiologic mechanisms, cellular response to immune reactions, improved techniques for diagnosis and prognosis and fundamental immunochemical reactions of significance in disease states. These studies are also pertinent to disorders involving autoimmunity, isoimmunization, and transplantation immunity; and to mechanisms of cellular secretion.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 51,001-24 CHB

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Study of Blood Coagulation and Diseases of Hemorrhage and Thrombosis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	N. Raphael Shulman	Chief	CHB NIADDK
Other:	Carol Kautz	Biologist	CHB NIADDK

COOPERATING UNITS (if any)

LAB/BRANCH
Clinical Hematology Branch

SECTION

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.25	1.0	.25

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Anion transport blocking agents were found to interfere with the clotting of fibrinogen by thrombin and with the attachment of thrombin to platelets. Platelet metabolic responses to thrombin were inhibited proportionally to the degree of inhibition of thrombin attachment, suggesting that platelet fibrinogen is the most likely receptor for thrombin and that the action of anion transport blockers on platelets does not support the "chemiosmotic" mechanism of cellular secretion.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 51002-09 CHB

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)
Mechanisms of Hormone and Transmitter Secretion

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Harvey B. Pollard	Senior Investigator	CHB NIADDK
OTHERS:	Mark A. Levine	Research Fellow	CHB NIADDK
	Janet H. Scott	Staff Fellow	CHB NIADDK
	Velia M. Fowler	Guest Worker	CHB NIADDK
	Oren Zinder	Visiting Scientist	CHB NIADDK

COOPERATING UNITS (if any)

Jack Cohen, LCP, NIADDK

LAB/BRANCH
Clinical Hematology

SECTION
Cell Biology and Biochemistry

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.25	5.00	0.25

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Exocytosis of Neurotransmitters, hormones and other cell secretory products is believed to occur by calcium-dependent movement of the secretory vesicle to the cell surface and subsequent fusion of the secretory vesicles with the plasma membrane. We have discovered a calcium-sensitive linkage of secretory vesicles to the cytoskeletal protein actin. We have found that the calcium binding protein synexin binds the inside of the plasma membrane as well as the secretory vesicle membrane. Membranes drawn together by this protein were demonstrated to fuse together when exposed to arachidonic acid at concentrations comparable to those present in stimulated cells. Working with isolated adrenal medullary cells we have found that epinephrine secretion appears to depend upon an energy dependent chemiosmotic lysis of the secretory vesicle.

ANNUAL REPORT OF THE GENETICS AND BIOCHEMISTRY BRANCH

In the area of molecular genetics, Dr. Camerini-Otero's continued studies of transformation of cultured mouse Ltk^- cells by viral thymidine kinase have shown that integration of the foreign DNA into the mouse cellular DNA requires areas of homology and possibly other structural features such as palindromic and inverted repeat sequences. In addition, he has shown, in collaboration with the laboratories of Dr. J. Seidman (NICHD) and of Dr. Singer (NCI) that several genes for histocompatibility antigens may be assayed by contransformations of Ltk^- cells, in which these genes direct the synthesis of protein products.

Dr. Zasloff studied the expression of a cloned variant gene for initiator methionine tRNA, which differs from the normal gene by one base substitution. The variant gene is transcribed in Xenopus oocytes; the resulting $tRNA_i^{met}$ is processed correctly but slowly, and fails to leave the nucleus. This is the first evidence for a selective mechanism for transport of tRNA from nucleus to cytoplasm.

In the area of biochemical genetics, Dr. Neufeld has identified a rare case of Tay-Sachs disease in which an altered, insoluble, α-chain of β-hexosaminidase is not transported through cellular organelles to lysosomes or to the exterior of the cell. The biosynthesis of the receptor for lysosomal enzymes has been investigated, as has its localization within the cell and on the cell surface (collaborative with Dr. Willingham, NCI). Dr. Robbins isolated novel mutants of Chinese hamster ovary cells which show pleiotropic defects of lysosomal function. One of these is defective in some early, not yet identified step of endocytosis, whereas the other is defective in its ability to glycosylate newly made proteins.

In clinical genetics, Dr. Zasloff is testing several synthetic retinoids for the treatment of fibrodysplasia ossificans progressiva (FOP). Dr. Neufeld, in collaboration with Dr. Shull (University of Tennessee), has identified a canine disease as a model for mucopolysaccharidosis I (Hurler/Scheie syndrome).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 52000-15 GBB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
The Biochemical Lesions in the Genetic Mucopolysaccharidoses and Mucolipidoses

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Elizabeth F. Neufeld	Biochemist & Branch Chief	GBB NIADDK
Other:	Gideon Bach	Visiting Scientist	GBB NIADDK
	Clara Hall	Research Chemist	GBB NIADDK
	Rachel Myerowitz	Staff Fellow	GBB NIADDK
	Christina Puchalski	Chemist	GBB NIADDK
	Richard Proia	Guest Worker	GBB NIADDK
	Gary Sahagian	Guest Worker	GBB NIADDK
	Elizabeth Spellacy	Guest Worker	GBB NIADDK

COOPERATING UNITS (if any)
University of Tennessee, Knoxville, TN (Dr. Robert Shull)
Laboratory of Molecular Biology, NCI/NIH (Drs. Willingham, Pastan)

LAB/BRANCH
Genetics and Biochemistry Branch

SECTION

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 8	PROFESSIONAL: 7.5	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The synthesis, processing and translocation of lysosomal enzymes and of their receptor are studied in the normal situation and in genetic disorders of lysosomal function. 1) The receptor is synthesized as a polypeptide of 215,000 daltons; it is glycosylated on asparagine residues and phosphorylated on serine residues. 2) Endocytosed enzyme enters a prelysosomal compartment, which can be separated on Percoll gradients. 3) Liver tissue of patients with I-cell disease have a normal level of many lysosomal enzymes in spite of a deficiency of the P-GlcNAc transferase which synthesizes the mannose 6-phosphate recognition marker. 4) Studies of the biosynthesis of β-hexosaminidase in a cell-free system as well as in intact fibroblasts have revealed a novel defect; in one Tay-Sachs patient, an altered insoluble α-chain is inserted into the endoplasmic reticulum and not transported further. 5) A heritable canine mucopolysaccharidosis has been identified and shown to be a good biochemical and clinical model for human α-L-iduronidase deficiency disease mucopolysaccharidosis I, Hurler/Scheie variant).

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AM 52002-05 GBB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
The Role of Cell-Surface Antigens in Development

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Rafael D. Camerini-Otero	Senior Surgeon	GBB	NIADDK
Other:	Richard A. Anderson	Staff Fellow	GBB	NIADDK

COOPERATING UNITS (if any)
Dr. Dinah Singer, Transplantation Biology Section, Immunology Branch, NCI
Dr. Jonathan Seidman, Gene Organization and Control Section, Laboratory of Molecular Genetics, NICHD

LAB/BRANCH
Genetics and Biochemistry Branch

SECTION

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0.4	PROFESSIONAL: 0.4	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Several genomic clones that hybridized to a cDNA clone for HLA (human histocompatibility) were isolated from pig and mouse genomic lambda libraries. Mouse tk^- cells were then cotransformed with herpes simplex virus thymidine kinase gene and several of these clones that putatively encoded for histocompatibility antigens. Several of these cotransformants appear to coexpress thymidine kinase and proteins that can be identified by the use of antisera to known histocompatibility antigens. Other genomic clones cannot be identified with such antisera; some probably cannot express any histocompatibility antigens and others probably express antigens unknown at the present time. Among those that express known antigens we have identified genomic clones encoding for an SLA (porcine major histocompatibility) antigen and those encoding for mouse major histocompatibility antigens H-2L and H-2D.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 52003-04 GBB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Structure of the Methionine Initiator tRNA Genes in the Human Genome

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Michael A. Zasloff	Senior Surgeon	GBB	NIADDK
Other:	Samuel Adeniyi-Jones	Visiting Associate	GBB	NIADDK

COOPERATING UNITS (if any)
Martin Rosenberg, Laboratory of Biochemistry, NCI
Dean Hamer, Laboratory of Biochemistry, NCI

LAB/BRANCH
Genetics and Biochemistry Branch

SECTION

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 2.5	PROFESSIONAL: 2	OTHER: .5

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER
☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Functional studies of two cloned non-allelic human methionine initiator tRNA genes were continued. One of the cloned genes, containing a variant sequence, was shown to direct the synthesis of a mature variant initiator tRNA met species following injection of either the cloned gene of primary transcript into the intact X. laevis germinal vesicle. Unlike the normal initiator tRNA met species which was efficiently transported from the oocyte nucleus, the newly synthesized variant initiator tRNA met accumulated within the nucleus, limited from escape by the nuclear membrane. Combined with our previous in vitro studies, the data demonstrate that while processing and transport of tRNA species are functionally linked, they nonetheless can be uncoupled within the intact cell; and they provide the strongest argument to date that the processing endonuclease may be a component in the tRNA transport mechanism.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 52006-03 GBB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Pathophysiology of Fibrodysplasia Ossificans Progressiva and Therapeutic Intervention

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Michael A. Zasloff	Senior Surgeon	GBB	NIADDK

COOPERATING UNITS (if any)
Michael Levine, Metabolic Disease Branch, NIADDK
Harri Reddi, Laboratory of Biological Structure, NIDR

LAB/BRANCH
Genetics and Biochemistry Branch

SECTION

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

CHECK APPROPRIATE BOX(ES)
☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Studies on the therapeutic utility of several synthetic retinoids in the treatment of fibrodysplasia ossificans progressiva (FOP) continues. Extensive review of clinical histories of affected females has revealed that primary and secondary amenorrhea is, as previously suspected, an associated abnormality in females affected with FOP.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 52007-03 GBB

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Pseudomonas Infection in Cystic Fibrosis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Michael A. Zasloff	Senior Surgeon	GBB	NIADDK
Other:	Carmen Tejedor	Guest Worker	GBB	NIADDK

COOPERATING UNITS (if any)

Laboratory of Biochemistry and Metabolism, NIADDK - Dr. John Foulds

LAB/BRANCH

Genetics and Biochemistry Branch

SECTION

INSTITUTE AND LOCATION

NIADDK,NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0	PROFESSIONAL: 0	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Project terminated.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AM 52008-03 GBB

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Gene Expression and Human Genetics

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Rafael D. Camerini-Otero	Senior Surgeon	GBB	NIADDK
Other:	Richard A. Anderson	Staff Fellow	GBB	NIADDK
	Michael Huberman	Guest Worker	GBB	NIADDK
	Shingo Kato	Visiting Fellow	GBB	NIADDK

COOPERATING UNITS (if any)

None

LAB/BRANCH

Genetics and Biochemistry Branch

SECTION

INSTITUTE AND LOCATION

NIADDK,NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.3	2.5	1.8

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We have been able to isolate as plasmids the products of recombination events occurring in mouse cell lines created by co-transferring unlinked pBR322 and Herpes Simplex Virus thymidine kinase gene (Tk) sequences into L cells deficient in thymidine kinase. The co-transferred DNAs were linked inside the eukaryotic cells and were integrated into high molecular weight cellular DNA. The plasmids were created by digesting DNA purified from the transformed cells with a restriction endonuclease and then ligating the resulting fragments under conditions favoring intra-molecular circularization. Plasmids were isolated by using the ligated DNA to transform E. coli and then selecting for ampicillin resistant colonies. Many of these "rescued" plasmids contain the pBR322 replication origin and BLA gene linked to Tk or cellular sequences or both. By using the cellular DNA sequences near the junctions as hybridization probes for genomic Southern blots we have shown that the DNA originates from unique sequence mouse DNA. Sequence analysis of the recombination junctions consistently showed that at least 15-20 BP homologies exist between the (otherwise grossly dissimilar) DNAs near the point where the linkage occurred. In addition, several palindromic and inverted repeat sequences appear to have been involved in the recombination process.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 52009-03 GBB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Chinese Hamster Ovary Cell Mutants with Altered Lysosomal Function

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	April R. Robbins	Senior Staff Fellow	GBB	NIADDK
	Clara W. Hall	Research Chemist	GBB	NIADDK

COOPERATING UNITS (if any)
Sharon S. Krag, Biochemistry Department, School of Public Health, Johns Hopkins University, Baltimore, Maryland; Constance Oliver, Laboratory of Biological Structure, National Institute of Dental Research, Bethesda, Maryland

LAB/BRANCH
Genetics and Biochemistry Branch

SECTION

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.6	1.5	.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In order to dissect the complex processes of receptor-mediated endocytosis and compartmentalization of acid hydrolases into lysosomes we have isolated mutant CHO cells defective in these functions: I) Endocytosis mutants were obtained by first selecting for cells resistant to diptheria toxin then screening the resistant cells for those unable to take up acid hydrolases. The phenotype of one of these mutants closely resembled that of normal cells following treatment with lysosomotropic amines; however, lysosomal acidification and transglutaminase activity, two targets of inhibition by the amines, appeared normal in this mutant II). Mutants simultaneously defective in α-L-iduronidase and β-glucuronidase were isolated in order to examine biosynthesis of the oligosaccharides on acid hydrolases; it is the phosphorylation of these oligosaccharides that distinguishes acid hydrolases from other glycoproteins. One of these mutants exhibited varying levels of oligosaccharide transfer, dependent upon the nature of the protein acceptor; i.e., differential glycosylation of the two polypeptides of β-hexosaminidase, and of the glycoproteins of two lytic viruses, Sindbis and vesicular stomatitis virus.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 52010-01 GBB

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Mechanism of Action of Thyroid Hormone

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Michael A. Zasloff	Senior Surgeon	GBB	NIADDK
Other:	Stuart A. Stein	Medical Staff Fellow	GBB	NIADDK

COOPERATING UNITS (if any)

Vera Nikodem, Clinical Endocrinology Branch, NIADDK

LAB/BRANCH

Genetics and Biochemistry Branch

SECTION

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 1.2	PROFESSIONAL: 1.2	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Studies on the mechanism of action of thyroid hormone at the gene level have begun. To this end we have begun to clone a library of cDNA species from rat liver removed from animals treated with pharmacologic doses of thyroid hormone. Initial studies of the mRNA preparation of this tissue has confirmed that at least one gene product, the mRNA for malic enzyme has been induced to levels expected from published accounts.

PHS-6040
(Rev. 2-81)

ANNUAL REPORT OF THE DIGESTIVE DISEASES BRANCH

SUMMARY OF BRANCH ACTIVITIES

The Digestive Diseases Branch has two sections (Section on Gastroenterology and the Liver Diseases Section). The Liver Diseases Section has 2 senior physicians and 3 medical staff fellows; the Section on Gastroenterology has 3 senior physicians and 5 medical staff fellows. The Digestive Diseases Branch also has 4 people in the NIH visiting program and 5-10 guest investigators from other laboratories.

Detailed summaries of the activities of each section precede the individual project reports. Both sections are engaged in investigations of basic biologic processes (e.g., hormone action, membrane transport, cellular and humoral immunology) and are attempting to apply this information to understand the pathophysiology of various disorders involving the liver and gastrointestinal tract. Both sections are also involved in attempts to improve therapy of clinical disorders such as neoplasms associated with overproduction of gastrointestinal hormones, hepatitis and fulminent hepatic failure.

Section on Gastroenterology

We are in the process of following more that 40 patients with Zollinger-Ellison syndrome (ZES, gastrin-producing neoplasm, hypergastrinemia and increased secretion of gastric acid). All patients are being managed using oral medication (cimetidine or ranitidine alone or plus an anticholinergic agent). During this past year we have found that over time virtually all patients require an increase in the frequency or amount of medication. We also found that in patients with ZES there is no correlation between gastric acid secretion and the presence of symptoms attributable to gastric hyperacidity. Thus, the adequacy of medication in inhibiting gastric acid secretion cannot be determined by assessment of symptoms but must be evaluated by measuring the extent of inhibition of gastric acid secretion. Of male subjects who are being treated with high doses of cimetidine (4 gm/day), approximately half develop impotence. This impotence can be reversed within a few weeks by replacing cimetidine with ranitidine - an H_2-receptor antagonist that lacks the anti-androgen activity of cimetidine. Many patients with ZES require an H_2-receptor antagonist plus an anticholinergic agent to achieve adequate inhibition of gastric acid secretion; however, in a significant number of patients the dose of anticholinergic agent is limited by unacceptable anticholinergic side-effects such as blurred vision, dry mouth or constipation. Pirenzepine, an anticholinergic agent with selective actions on secretory tissues, can be used in patients with ZES without producing untoward side-effects.

In pancreatic acinar cells a number of secretagogues act by causing mobilization of cellular calcium. During this past year we have found that acinar cells possess a phospholipid-requiring, calcium-dependent protein kinase that causes phosphorylation of several intracellular proteins, two of which appear to be related to enzyme secretion.

Two years ago we found that proglumide, a derivative of glutaramic acid, is a selective, competitive antagonist of the interaction of cholecystokinin with its receptors on pancreatic acinar cells. During this past year we have shown that proglumide also antagonizes the interaction of cholecystokinin with its receptors on small intestinal smooth muscle cells and on gastric chief cells.

Previously, we showed that certain derivatives of cyclic nucleotides (e.g., dibutyryl cyclic GMP) as well as proglumide and benzotript, are specific cholecystokinin-receptor antagonists. During this past year we discovered a third class of cholecystokinin-receptor antagonists - the C-terminal tripeptide of cholecystokinin. Fragments as short as the C-terminal dipeptide of cholecystokinin, if used in sufficiently high concentrations, also function as specific receptor antagonists.

During this past year we have developed a new *in vitro* preparation that can be used to explore the cellular basis of action of gastrointestinal peptides - dispersed glands prepared from rat or rabbit gastric mucosa. These cells can be used to measure accumulation of weak bases (an index of gastric acid secretion) or to measure pepsinogen secretion. We found that pepsinogen secretion from gastric glands is stimulated by cholecystokinin and structurally related peptides, muscarinic cholinergic agents, as well as secretin and structurally related peptides. Cholecystokinin and cholinergic agents appear to stimulate pepsinogen secretion by causing mobilization of cellular calcium whereas secretin stimulates enzyme secretion by causing activation of adenylate cyclase and increased cellular cyclic AMP. Potentiation of pepsinogen secretion occurs when a secretagogue that mobilizes cellular calcium is combined with a secretagogue that increases cyclic AMP.

Three years ago we showed that bombesin and structurally related peptides cause desensitization of pancreatic enzyme secretion *in vitro* and that this desensitization is specific for those peptides that interact with the bombesin receptor. During this past year we have shown that bombesin causes desensitization by causing accumulation of inactive bombesin receptors within the pancreatic acinar cell. In contrast to a number of other systems, bombesin-induced receptor inactivation is insensitive to lysomotrophic agents such as chloroquine.

Liver Diseases Section

The Liver Diseases Section is currently conducting nine principal studies:

I. Studies of the Pathogenesis of Encephalopathy in Fulminant Hepatic Failure

A highly reproducible model of fulminant hepatic failure (FHF) has been developed in an inbred strain of rabbits. The model resembles human FHF in all important respects.

The pattern and amplitude of visual evoked potentials in the central nervous system have been studied in rabbits. These potentials reflect post-synaptic neuronal activity. The recording of visual evoked potentials has been shown to be a reliable, quick, non-invasive method of quantifying neurologic changes during the evolution of hepatic encephalopathy (HE). Furthermore, the pattern of visual evoked potentials associated with HE is similar to that found in the postictal state and coma induced by barbiturates and benzodiazepines, but differs fundamentally from that associated with ether-induced coma. As barbiturates and benzodiazepines are known to mediate their neuroinhibitory effects by interacting with post-synaptic receptors for γ-aminobutyric acid (GABA) in the central nervous system, putative encephalotoxins in HE might mediate neural inhibition by interacting with the GABA inhibitory neurotransmitter system.

GABA metabolism and the status of the GABA neurotransmitter system have been investigated in HE. It has been shown that (i) A major source of GABA in peripheral blood is the enteric bacterial flora; (ii) plasma levels of GABA are increased by more than an order of magnitude in FHF but not in another type of metabolic encephalopathy; (iii) as the liver fails plasma levels of GABA become elevated before the onset of HE; and (iv) the density of receptors for GABA on postsynaptic neural membranes of the brain is increased in FHF. To determine whether GABA in plasma can gain access to the brain, the transport of the non-metabolizable isomer of GABA, α-aminoisobutyric acid (AIB), across the blood brain barrier was measured by a computer-assisted quantitative autoradiographic technique. Blood to brain transport of ^{14}C-AIB is minimal in normal rabbits but with liver failure it increases markedly in specific gray areas of the brain prior to the onset of HE. These findings suggest that as the liver fails plasma GABA derived from the gut crosses an abnormally porous blood-brain barrier before the onset of HE and contributes to the neural inhibition of HE. The increase in the number of GABA receptors in HE may be associated with enhanced sensitivity of the brain to GABA-ergic neural inhibition.

The number of binding sites for benzodiazepines on postsynaptic membranes is also increased in HE, suggesting that the benzodiazepine binding site and the GABA receptor are regulated as a single operational unit and that the increased sensitivity to benzodiazepines in HE is due to the presence of more benzodiazepine receptors permitting increased drug effect.

Clearance of ^{3}H-GABA from plasma is markedly decreased in liver failure, primarily due to inefficient hepatic extraction. Studies with isolated hepatocytes indicate that GABA binds specifically to the high affinity acceptor of the A amino acid transport system. Thus, impaired function of this transport system in liver failure could contribute to increased plasma GABA levels in HE.

Studies of the binding of GABA to post-synaptic membranes prepared from brains obtained at human autopsies and measurements of plasma GABA levels in patients with a variety of liver and other diseases are being conducted to determine whether the findings obtained with the rabbit model of FHF apply to man. Preliminary results indicate that the number of GABA receptors is increased in the brains of patients dying of FHF or chronic hepatocellular failure but not in patients dying from a variety of other causes. Furthermore, plasma GABA levels were found to be elevated fourfold in patients with decompensated cirrhosis without overt HE but were elevated more than tenfold in patients with cirrhosis complicated by HE or a gastrointestinal hemorrhage or with FHF.

Hyperammonemic encephalopathy differs fundamentally from HE in terms of the associated changes in visual evoked potentials and receptors for the excitatory neurotransmitter glutamate. In HE the number of glutamate receptors decrease without change in affinity whereas in hyperammonemia both the number and affinity of these receptors increase. L-leucine can prevent ammonia-induced changes in visual evoked potentials and in glutamate receptors. These findings suggest that hyperammonemia is not a good model of HE and that infusions of branch chain amino acids may counteract ammonia-induced changes in neurotransmission. Further studies of the status of neurotransmitter receptors have indicated that in HE there is a selective increase in receptors for inhibitory amino acid neurotransmitters, a selective decrease in receptors for excitatory amino acid neurotransmitters and no change in receptors for acetylcholine, opiates and enkaphalins. Thus, increased sensitivity to inhibitory neurotransmitters and decreased sensitivity to excitatory neurotransmitters may contribute to the neural inhibition of HE.

HE is associated with an increase in the lipid:protein ratio of synaptic plasma membranes without associated changes in membrane fluidity or major membrane proteins. The altered composition of synaptic plasma membranes in HE may be the basis of changes in membrane function, including the mediation of neurotransmission, in this syndrome. [E. A. Jones, S. C. Pappas, P. Ferenci, J. G. Waggoner, P. Skolnick (NIADDK), P. Munson (NICHHD), J. T. Fenstermacher (NCI)].

II. Studies of Cellular Immune Function in Primary Biliary Cirrhosis

The role of abnormal immune mechanisms in the pathogenesis and perpetuation of the hepatobiliary lesion of primary biliary cirrhosis (PBC) is being studied. Recent studies have been designed to determine whether there is a defect in immune regulation in patients with PBC. T lymphocyte-mediated help and suppression of pokeweed mitogen-induced immunoglobulin (Ig) synthesis by cultured B lymphocytes have been quantitated in vitro using sensitive radioimmunoassays to measure IgG and IgM. Ig synthesis by cultures containing different T:B cell ratios, cultures containing B cells in the presence of autologous or allogeneic T cells, and cultures containing B cells and unirradiated or irradiated T cells was determined. The results suggest that there is deficient suppression of Ig synthesis in PBC due to a T cell defect. Patient control experiments indicated that the suppressor T cell defect in PBC is not secondary to chronic cholestasis or hepatocellular disease per se and that the defect exhibits specificity for PBC among liver diseases.

It has been shown that T cells can be induced to proliferate when cultured with either autologous or allogeneic irradiated B cells. These phenomena are known as the autologous and allogeneic mixed lymphoyte reactions (MLR), respectively. The recent finding by others that patients with systemic lupus erythematosus (SLE) exhibit deficiencies of both suppressor T cell function and the autologous MLR prompted studies of the MLR in PBC. For patients with PBC, the mean autologous but not allogeneic MLR was found to be significantly less than that for controls. Thus, as in SLE, deficiencies of both suppressor T cell function and the autologous MLR are found in PBC. These deficiencies could be due to a common defect of cell interactions involved in the generation of suppressor effector cells. Such a defect could play an important role in the pathogenesis of PBC by providing an abnormal immunologic environment which predisposes to the development of autoimmune phenomena and a state of immune hyperresponsiveness.

In additional studies the proliferative responses of peripheral blood mononuclear cells (PBMC) and T cells when cultured with hapten-modified stimulator cells were measured. The stimulator cells were PBMC or B cell-enriched PBMC that had been treated with trinitrobenzenesulfonic acid and irradiated. The results indicated that the primary but not the secondary proliferative response is decreased in PBC. They suggest that in PBC there is a decreased proliferative response of a subpopulation of suppressor T cells which may be responsible for a decreased recognition of exogenous antigens in association with autologous cell surface antigens.

Cytotoxicity mediated by circulating natural killer (NK) cells is reduced in PBC. This phenomenon is not due to a reduced number of effector cells, inhibitory serum factors or suppressor cells. The defect can be partially corrected by incubating PBC NK cells with interferon.

Complement receptor function in PBC Has been assessed by quantitating the ability of peripheral blood monocytes to form rosettes with complement-coated sheep erythrocytes. PBC monocytes have a normal capacity to form rosettes but PBC serum in the presence or absence of normal serum inhibits rosette formation. This inhibition is mediated by PBC IgM and not IgG and does not depend on complement. Thus, the previously demonstrated defective complement receptor mediated clearance function in PBC appears to be due to the presence of an IgM antibody which blocks complement receptors on macrophages. [E. A. Jones, S. P. James (NCI), G. Y. Minuk, C. O. Elson (NCI), and W. Strober (NCI)].

III. Studies of Protease Inhibitor Phenotypes

Pi phenotypes of sera from a large number of black South African patients with hepatocellular carcinoma and from age and sex matched healthy black South Africans are being determined to assess whether the incidence of any particular Pi phenotypes is increased in the patient population. [E. A. Jones and J. Vergalla].

IV. Studies of Chronic Hepatitis B Surface Antigen (HBsAg) Carriers

One hundred and fifty HBsAg-positive patients with chronic liver disease of varying degrees of severity have been enrolled in this project. Spontaneous complete resolution of chronic type B hepatitis, with loss of serum HBsAg, occurs in about 1% of patients per year but spontaneous remissions in disease activity occur in about 14% of patients per year. These remissions are characterized by disappearance of serum HBV-DNA and DNA polymerase reactivity, seroconversion from HBeAg to anti-HBe and a fall in elevated serum aminotransferase levels to values within the normal range.

A randomized controlled trial of a 28 day course of the anti-viral drug adenine arabinoside 5' monophosphate (Ara-AMP) has been conducted in 20 patients with HBeAg positive chronic type B hepatitis. Ara-AMP led to a dramatic and usually complete inhibition of serum levels of DNA polymerase and HBV-DNA. However, when therapy was stopped, the levels of these viral markers returned to pretreatment values in most patients. Three of 10 treated patients and 2 of 10 non-treated patients have exhibited a long lasting remission of disease activity (as defined above). The most troublesome side effect of Ara-AMP was severe muscle pains lasting up to 7 weeks after stopping the drug.

Fifteen patients with chronic type B hepatitis were receiving corticosteroids when referred; all were positive for serum HBV-DNA, DNA polymerase and HBeAg. None seroconverted from HBeAg to anti-HBe while on corticosteroids. When corticosteroids were withdrawn 7 of the 15 seroconverted from HBeAg to anti-HBe within six months. These observations indicate that in patients with chronic type B hepatitis corticosteroids promote viral replication and prevent seroconversion from HBeAg to anti-HBe and that the withdrawl of corticosteroids may promote seroconversion from HBeAg to anti-HBe. Furthermore, in patients with malignant disease who are carriers of HBsAg, immunosuppressive chemotherapy frequently leads to reactivation of chronic HBV infection while stopping such therapy is frequently followed by an acute hepatitis and subseqent loss of all HBV markers. To determine if withdrawal of immuno-suppressive therapy is associated with a higher incidence of remissions in disease activity than occurs spontaneously, a controlled trial of a one-month high dose course of prednisolone for patients with chronic type B hepatitis has been initiated.

Lymphocytes from the majority of patients with chronic type B hepatitis undergo normal proliferative responses to mitogens and are capable of synthesizing normal amounts of immunoglobulins in culture. The ability of circulating NK cells in these patients to mediate cytotoxicity is increased.

However, lymphocytes from these patients are consistently unable to synthesize detectable amounts of anti-HBs in culture. This defect is specific for anti-HBs and is presumably due to a lack of precursor B cells capable of specifically synthesizing this antibody. This defect is often associated with a nonspecific increase in suppressor T cell activity.

The ability of HBsAg to mediate delayed hypersensitivity when administered intradermally and induce blastogenesis in lymphocytes in vitro has been tested in patients with chronic type B hepatitis, control HBsAg and anti-HBs negative individuals and anti-HBs positive individuals who have recovered from type B hepatitis. The results indicate that HBsAg is not a potent inducer of delayed hypersensitivity in man. This property of HBsAg may be a contributing factor in the development of chronic type B hepatitis. [J. H. Hoofnagle, S. Straus (NIAID), G. Y. Minuk, W. G. E. Cooksley, J. Gerin (Georgetown University), R. Johnson (NIAID), R. H. Purcell (NIAID), and E. A. Jones].

V. Controlled Trial of Chlorambucil Therapy in Primary Biliary Cirrhosis

Primary biliary cirrhosis (PBC) is characterized by slowly progressive intrahepatic cholestasis due to a chronic non-suppurative destructive cholangitis involving the septal and larger interlobular bile ducts. When symptomatic the disease appears to be uniformly fatal. Trials of corticosteroids and azathioprine have not shown that either of these agents effectively inhibits the progression of the disease or increases survival. Trials of D-penicillamine have yielded conflicting data with respect to the effect of the drug on the progression of the disease and survival. Because certain other chronic inflammatory autoimmune diseases appear to respond favorably to alkylating agents but not to azathioprine, a controlled randomized trial of an alkylating agent for the treatment of PBC has been initiated. The alkylating agent chosen for the trial was chlorambucil rather than cyclophosphamide because of the less severe toxicity associated with the use of the former drug. So far 24 patients have been admitted to the trial; 13 have been randomized to the treatment group and 11 to the control group. The dose is titrated so that a polymorphonuclear leucocyte count of at least 1000/mm^3 is maintained while the lymphocyte count is decreased to about 50% of pretreatment values. During follow-up no patient has died. Chlorambucil therapy has been discontinued in two patients. Twelve patients (7 treated and 5 untreated) have been followed for 2 years. Three of the 5 untreated have had significant complications of their liver disease and in 2 of the 5 untreated patients serum bilirubin levels have doubled. In contrast none of the 7 treated patients have had significant complications of their liver disease and serum bilirubin levels in these patients have not increased by more than 25%. Liver biopsies after one and two years of treatment have revealed a decrease in inflammatory activity. However, it is two early to determine whether chlorambucil therapy mediates a definitive beneficial effect on the course of PBC. [E. A. Jones, J. H. Hoofnagle, G. Y. Minuk, S. C. Pappas, R. Hanson].

VI. Studies of Chronic Non-A, Non-B Hepatitis

Patients with well-defined chronic non-A, non-B hepatitis are being evaluated. The natural history of this condition is being studied in collaboration with Dr. Harvey J. Alter (NIH Clinical Center Blood Bank). Twenty-eight patients with HBsAg negative post-transfusion hepatitis whose serum levels of aminotransferases remained elevated for more than six months have been evaluated and have undergone percutaneous liver biopsy. These patients have had evidence of hepatitis for periods varying between 1 and 10 years. Six experience mild degrees of lethargy or malaise; the remainder have no symptoms attributable to liver disease. Serum aminotransferase levels fluctuate from normal values to as high as 700 IU/l. The disease is not associated with an increased incidence of any HLA types. Liver biopsies have revealed chronic active hepatitis in 20 patients, chronic persistent hepatitis in 6 and alcoholic liver disease in 2. Six patients have compensated cirrhosis. Follow-up liver biopsies from 13 patients have demonstrated progression of the liver disease in 4, and milder degrees of hepatocellular disease in the rest.

Immunofluorescence studies on the liver biopsies using sera from patients who have recovered from non-A, non-B hepatitis have not revealed any potential non-A, non-B antigens. Furthermore, electron microscopy of the biopsies has not demonstrated viral antigens or definite viral particles in the liver biopsies. However, 50% of biopsies have shown peculiar intranuclear clusters of pleomorphic particles. Similar structures have also been seen in liver biopies from chimpanzees with non-A, non-B hepatitis. The significance and specificity of these nuclear changes are being investigated. In the course of assaying for antigens and antibodies specific for non-A, non-B hepatitis agar gel diffusion studies of sera from these patients have demonstrated a precipitin system similar to that described by other workers in this field. However, evaluation of this system has demonstrated that it is not due to an antigen-antibody reaction and that it is found in liver diseases other than non-A, non-B hepatitis.

Sixty-three blood donors with raised serum ALT levels have been evaluated. Of the approximately 80% of these donors who have persistently elevated serum ALT levels, 12 have undergone percutaneous liver biopsy. This procedure has revealed hemochromatosis in one patient, alcoholic hepatitis in two, non-alcoholic steatonecrosis in two, chronic persistent hepatitis in five and chronic active hepatitis in one. One biopsy was considered normal. This study will provide a framework for recommendations for follow-up and evaluation of asymptomatic patients whose serum ALT levels are chronically elevated. [J. H. Hoofnagle, H. J. Alter (CC:BB), E. A. Jones, S. C. Pappas, R. G. Hanson, R. H. Purcell (NIAID), G. L. Davis, S. M. Feinstone (NIAID), K. Ishak (A.F.I.P.), G. Y. Minuk].

VII. Studies of the Uptake of Lipoproteins by Hepatic Cells

Familial high density lipoprotein (HDL) deficiency or Tangier disease is characterized by low levels of plasma HDL and apolipoproteins (apo) A-I and A-II, rapid catabolism of apo A-I and A-II and cholesterol laden reticuloendothelial (RE) cells. Furthermore, in Tangier disease apo A-I and A-II are predominantly associated with lipoprotein particles of density less than 1.063 g/ml rather than HDL. The mechanisms underlying these abnormalities of lipoprotein metabolism in Tangier disease have been investigated. Rat endothelial, Kupffer and parenchymal cells were isolated by in situ collagenase liver perfusion, centrifugation on Percoll gradients and centrifugal elutriation. Apo A-I and A-II were isolated from normal subjects and from patients who were homozygotes for Tangier disease. After apoproteins were radioiodinated they were reassociated with lipoprotein particles of density less than 1.063 g/ml from serum of normal subjects and of patients with Tangier disease. Uptake of these labeled particles by hepatic cells in vitro was quantitated. The results obtained indicate that the increased RE cell uptake of cholesterol in Tangier disease is probably mediated by enhanced uptake of Tangier apo A-I by RE cells and that the rapid clearance of lipoproteins from the plasma in Tangier disease can be accounted for by hepatic parenchymal cell uptake of Tangier apo A-I. This study provides evidence that a structurally abnormal apo A-I mediates both the rapid catabolism of lipoproteins and the RE cell accumulation of cholesterol in Tangier disease. [E. A. Jones, J. Vergalla, E. J. Schaefer (NHLBI), and H. B. Brewer Jr. (NHLBI)].

VIII. Studies of the Uptake of Glycoproteins by Hepatic Cells

The cellular localization and carbohydrate specificities of a glycoprotein recognition system on rat hepatic sinusoidal cells ahve been investigated. Rat Kupffer cells and endothelial cells were isolated by in situ collagenase liver perfusion, centrifugation on Percoll gradients and centrifugal elutriation. ^{125}I-labeled agalactoorosomucoid (AGOR), an N-acetylglucosamine-terminated glycoprotein was selectively taken up by hepatic endothelial cells but not Kupffer cells in vitro. Uptake was shown to be protein dependent, calcium ion dependent and saturable and could be described by Michaelis-Menten kinetics. Uptake was inhibited not only by N-acetylglucosamine, mannose and mannan but also by glucose, fructose and a glucose-albumin conjugate. Thus, there is a glycoprotein recognition system on hepatic endothelial cells which is glucose sensitive. The existence of such a receptor suggests a mechanism for abnormal glycoprotein metabolism in diabetes mellitus. [E. A. Jones, J. Vergalla].

IX. Studies on the Pathogenesis of Fibrosis Following Hepatocellular Injury

Studies are being designed to explore factors involved in hepatic fibrogenesis which is an important feature of many chronic liver diseases including cirrhosis. Recent studies, in which the types of collagen present in schistosomiasis-induced hepatic fibrosis in rabbits have been analyzed, indicate that smooth muscle cells (SMC) may be responsible for the syntehsis of fibrous tissue rather than hepatocytes. Furthermore, SMC have been shown to respond to specific factors by directed migration (chemotaxis). These observations raise the possibility that fibrosis arising in cirrhotic liver could be due to an invasion of the liver by connective-tissue derived cells, such as SMC, in response to the elaboration and release of chemoattractants in the liver. Using measurements of the degree of migration of SMC through collagen V coated filters as an index of chemotaxis, chemoattractants have been detected in conditioned media from cultures containing normal rat Kupffer cells, but not from those containing hepatocytes or hepatic endothelial cells. They were not detected in sonicated Kupffer cells or in conditioned media from cultures of Kupffer cells treated with inhibitors of protein synthesis. Chemoattractants were also detected in supernatants of cultures of human monocytes isolated from chronic type B hepatitis blood but not from normal blood. These findings are consistent with the hypothesis that hepatic fibrogenesis can arise as a result of collagen synthesis by modulated SMC after their migration to the liver in response to the release of chemoattractants. [E. A. Jones, W. G. E. Cooksley, C. McIvor, G. R. Martin (D:LDBA)].

Digestive Diseases Branch
Phoenix Clinical Research Section

Research at the Phoenix Clinical Research Section (PCRS) has been in three major areas: (1) noninsulin dependent diabetes mellitus, (2) obesity and energy balance and (3) lipoprotein metabolism. Investigations in these areas in the past year has been approached both by *in vivo* and *in vitro* techniques.

Diabetes Mellitus

Noninsulin dependent diabetes mellitus is most often characterized by abnormal insulin secretion in response to glucose challenge and by resistance to the hypoglycemic effects of insulin. *In vivo* studies completed at PCRS this past year have shown that compared to age and weight matched Caucasians the obese Pima Indians are more insulin resistant as measured by the steady state plasma glucose technique. Also, the obese glucose tolerant Pimas were less insulin resistant than weight matched, glucose intolerant Pima Indians. These data suggested that insulin resistance may be the major abnormality resulting in the high prevalence of diabetes mellitus in the Pimas; and also that obesity per se was not the only cause of the insulin resistance.

These data correlated well with the insulin secretory data. The Pima Indians had higher plasma insulin and C-peptide concentrations after oral or intravenous glucose than did Caucasians even after controlling for plasma glucose concentrations, age and obesity. Furthermore, the hyperinsulinemia was shown to be unrelated to any alterations in gastric emptying times.

The hyperinsulinemia of those obese Indians was further investigated in vivo by studying the suppressibility of beta cell secretion by several doses of exogenously infused insulin while maintaining euglycemia. The obese subjects had similar suppressibility of beta cell secretion when expressed as a percent of basal plasma C-peptide concentration, but at all levels of plasma insulin the absolute concentration of plasma C-peptide was higher in the obese subjects. This was consistent with a greater number and/or a higher secretory activity of beta cells in the obese subjects.

The data on insulin resistance and insulin secretion did not resolve the question of whether the hyperinsulinemia was primary and led to insulin resistance as a protective mechanism (against hypoglycemia) or whether the insulin resistance was primary and resulted in hyperinsulinemia to maintain normoglycemia. To help resolve this issue and to elucidate the cellular mechanisms of insulin resistance *in vitro* studies in cultured fibroblasts as well as in monocytes were completed. Both studies in Caucasians and Pimas, showed that there was a positive correlation between the body mass index (body weight (Kg)/height^2(cm^2)) and decreases in insulin receptor binding. This suggested that the insulin resistance of obesity could be a reflection of genetic differences which are expressed at the site of the insulin receptor. Subsequent studies on cellular insulin action however showed that there were no differences between fibroblasts from diabetic and non-diabetic Pimas. Therefore, the fibroblast studies were stopped and a different cell was studied, the adipocytes as this cell is a more likely target tissue for insulin action *in vivo*.

Methods for measuring insulin binding, glucose transport, glucose metabolism and lipolysis in human dipocytes (isolated from open abdominal wall biopsies) were developed. There were no differences in insulin binding or in the sensitivity of transport comparing obese glucose tolerance to obese diabetic Pima Indians. However, in the diabetics the maximum glucose transport rate was 69% of that observed in the glucose tolerance group. Furthermore, this defect was reversible after four weeks of insulin therapy, and correlated with changes observed in insulin mediated glucose disposal in vivo. These data suggested that, at least at the adipocyte cellular level, the diabetic subject did not have a inherited cellular defect of glucose transport. However, metabolic studies of those adipocytes reflect not only inherited characteristics but also reflect the metabolic milieu at the time of the biopsy. To more clearly examine genetically determined metabolic characteristics, a pre-adipocyte cell culture system was developed, based on previous knowledge from fibroblast cell culture techniques. The techniques for measuring glucose transport in this cell line have been developed and this system should provide important answers concerning genetically determined adipocyte metabolic characteristics. In addition, both the adipocyte and pre-adipocyte studies will be used to further clarify the mechanisms controlling glucose transport in those cells from both normally glucose tolerant and diabetic humans.

In addition to pursuing the pathogenetic mechanisms in noninsulin dependent diabetes mellitus, several studies of the effect of various therapies have been completed. Previous work here had shown increased insulin secretion and improved glucose tolerance after weight reduction in these diabetic Indians. This past year the effects of aerobic training and of short and long term insulin therapy have been examined. Aerobic training consistently increased early insulin secretion after oral glucose challenge but had incumbent effects on total glucose disposal as measured by the euglycemic clamp technique. Short-term (10 days) insulin therapy improved insulin secretion in these diabetic subjects but had no effect on in vivo insulin-mediated glucose disposal. Four weeks of insulin therapy however, improved insulin secretion, as well as insulin sensitivity at the level of the liver and the peripheral tissues. There also was a significant reduction in the basal euglycemic glucose production. The improvements persisted for two weeks after withdrawal after insulin treatment.

Our future plans for studies in the area of noninsulin dependent diabetes mellitus are to undertake a longitudinal study of "prediabetic" Pima Indians compared to non "prediabetic" Pima Indians. These groups can be defined as a result of the epidemiologic studies over the past 15 years. The ("prediabetic") offspring of a diabetic mother (age 18-45), with BMI>300 have a 30% incident rate of diabetes mellitus over 5 years, compared to a 5% incident rate in the (non "prediabetic") offspring of nondiabetic parents. By studying twice two groups of parents on a yearly basis for 5 years we hope to know the sequence of metabolic events that lead to the development of noninsulin dependent diabetes mellitus. We will be studying in vivo insulin secretion insulin sensitivity and responsiveness, as well as comparing these data to in vitro studies of their adipocytes and preadipocytes.

Obesity

Ever since it was found that the Pima Indians eat a diet that is similar to U.S. Caucasians in total calories and composition, their obesity has remained unexplained. One theory, as originally proposed by Neel is that the Pima Indians have a "thrifty gene" that makes them more efficient in storing energy from ingested nutrients (i.e. a decreased energy expenditure for ingested energy). In the past year this hypothesis has begun to be tested in collaboration with the University of Vermont. The responses to three thermogenic stimuli were tested--food, norepinephrine and exercise. These responses were assessed during the three nutritional states of weight maintenance, overfeeding and underfeeding; and were compared to similar studies in obese Caucasians (done at the University of Vermont). The results from the obese Pimas as well as the lean and obese Caucasians are currently being analyzed.

Along with these *in vivo* measurementsof energy expenditure studies have also been completed on cellular mechanism of thermogenesis. Diminished sodium-pump activity has been observed by us in intact red cells and isolated erythrocyte membranes from obese Pima Indian subjects. Isolated abdominal adipocytes had diminished insulin stimulation of sodium-pump activity in obese compared to lean subjects. No difference were observed, however, in the basal or insulin stimulated sodium-pump activity in diploid fibroblasts. Thus the diminished sodium-pump activity may not be an inherent thermogeneic defect, but may be a manifestation of the insulin resistant state which contributes to the development of obesity via decreased cellular thermogenesis.

Also, at the cellular level the techniques for estimating triglyceride accumulation rates in cultured pre-adipocytes are being developed. These measurements will help determine whether those are genetically determined differences in the adipocytes ability to accumulate triglyceride as a possible contributory factor to the development of obesity.

Lipoproteins

To determine if diabetes and obesity are associated with changes in serum lipoproteins and to delineate the relationship between serum lipoprotein concentrations and the pathogenesis of ischemic heart disease, serum lipoprotein concentrations (of all subclasses) have been measured in a large cohort of Pima Indians in collaboration with the Epidemiology and Field Studies Branch.

Despite the high prevalence of obesity in the Pimas, plasma total and LDL cholesterol concentrations in males over 25 and in all females were lower than Caucasian norms. The lower cholesterol in adult Pima males appears to be related to the lack of increase with age after age 25. In contrast, in females cholesterol concentrations increase with age but are lower than Caucasians at all ages. Both male and female Pima have higher HDL cholesterol than their Caucasian counterparts. Distribution of HDL subfractions were similar in male and female Pimas with no increase in HDL_2 levels in females as reported in other studies.

There was a strong negative correlation between obesity and HDL cholesterol in the Pimas. Triglyceride concentrations were positively correlated with obesity. Total and LDL cholesterol had no relation to body weight in the Pimas.

In the diabetics, there were increased levels of triglycerides, but increased levels of total and HDL-L cholesterol were observed only in females. The diabetics also had a higher incidence of cardiovascular disease.

EKG abnormalities were associated with lower HDL cholesterol and higher ratios of total/HDL cholesterol in those subjects. These data suggested that the hyperglycemia associated with diabetes may be influential in the development of its macrovascular complication.

In addition studies of lipoprotein metabolism were completed in diabetic and non-diabetic Pima Indians. The increased VLDL-TG in Pima diabetics appeared due to an overproduction. This may have been due to low free fatty acid concentrations in the Pima subjects. There also appeared to be a racial difference in adipose tissue lipoprotein lipid, i.e., the Pimas had lower activities than matched Caucasians. Furthermore, despite the known resistance to insulin's hypoglycemic effect in obesity, there appeared to be a normal sensitivity to insulin's anti-lipolytic actions.

Studies of the control of LDL-cholesterol metabolism have also been started by studying the metabolism of VLDL-apoB and LDL-apoB using autologous ^{131}-I-VLDL and ^{125}-I-LDL. Preliminary results suggest that there is a large shunt pathway for VLDL-apoB (away from LDL) in the Pimas that may account for there low plasma cholesterol.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 53001-12 DDB

PERIOD COVERED
October 1, 1981 to Sept. 30, 1982

TITLE OF PROJECT (80 characters or less)

Studies of Membrane Function

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. D. Gardner	Chief, Section on Gastroenterology	DDB, NIADDK
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COOPERATING UNITS (if any)

LAB/BRANCH
Digestive Diseases Branch

SECTION
Section on Gastroenterology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 5.2	PROFESSIONAL: 3.6	OTHER: 1.6

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The broad categories which are included in the project are: 1) Characterizing functionally the mechanism by which various substrates cross the plasma membrane of different mammalian cells; 2) identifying the metabolic and humoral factors which influence the transport of various substrates across the plasma membrane; 3) developing techniques which will distinguish between binding of a substrate to the membrane and translocation of the substrate across the membrane; 4) characterizing the mechanism by which the membrane transport of various substrates is altered in certain diseases; and 5) relating these alterations of membrane transport to the pathogenesis and clinical manifestations of the disease.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 53002-10 DDB

PERIOD COVERED
October 1, 1981 to Sept. 30, 1982

TITLE OF PROJECT (80 characters or less)

Gastrointestinal Hormones

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. D. Gardner	Chief, Section on Gastroenterology	DDB, NIADDK
OTHERS:	R. T. Jensen	Senior Investigator	DDB, NIADDK
	S. J. Pandol	Clinical Associate	DDB, NIADDK
	J-P. Bali	Guest Worker	DDB, NIADDK
	H. Adachi	Visiting Scientist	DDB, NIADDK
	J-P. Raufman	Clinical Associate	DDB, NIADDK
	G. Z. Pan	Visting Fellow	DDB, NIADDK
	M. Villanueva	Visiting Scientist	DDB, NIADDK
	H. Allende	Visiting Scientist	DDB, NIADDK
	B. M. Bissonnette	Clinical Associate	DDB, NIADDK
	J. M. Howard	Visiting Scientist	DDB, NIADDK
	K. E. McArthur	Clinical Associate	DDB, NIADDK
	M. Spanarkel	Clinical Associate	DDB, NIADDK

COOPERATING UNITS (if any)
Dept. of Chemistry, Case-Western Reserve Univ., Cleveland, Ohio
Div. of Cellular Biology, Kennedy Institute for Rheumatology, London, England

LAB/BRANCH
Digestive Diseases Branch

SECTION
Section on Gastroenterology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 5.6	PROFESSIONAL: 4.0	OTHER: 1.6

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In vitro systems are being used to study the mechanism of action of gastrin, secretin, cholecystokinin, bombesin, substance P and vasoactive intestinal peptide with their specific membrane receptors.

Clinical investigations are directed toward developing alternative forms of therapy for and elucidating the pathogenesis of disorders characterized by ectopic production of gastrointestinal hormones (e.g., Zollinger-Ellison syndrome and pancreatic cholera).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 53004-10 DDB

PERIOD COVERED
October 1, 1981 to Sept. 30, 1982

TITLE OF PROJECT (80 characters or less)
Cyclic Nucleotide Mediated Functions

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. D. Gardner	Chief, Section on Gastroenterology	DDB, NIADDK
OTHERS:	R. T. Jensen	Senior Investigator	DDB, NIADDK
	M. J. Collen	Senior Investigator	DDB, NIADDK
	J-P. Raufman	Clinical Associate	DDB, NIADDK
	M. Villanueva, H. Adachi	Visiting Scientists	DDB, NIADDK
	G. Z. Pan	Visiting Fellow	DDB, NIADDK
	B. M. Bissonnette	Clinical Associate	DDB, NIADDK
	M. Jackson, J-P. Bali	Guest Workers	DDB, NIADDK
	T. Q. Garvey	Guest Worker	DDB, NIADDK
	H. Allende	Clinical Associate	DDB, NIADDK
	J. M. Howard	Visiting Scientist	DDB, NIADDK
	K. E. McArthur	Clinical Associate	DDB, NIADDK
	M. Spanarkel	Clinical Associate	DDB, NIADDK

COOPERATING UNITS (if any)

LAB/BRANCH
Digestive Diseases Branch

SECTION
Section on Gastroenterology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 6.6	PROFESSIONAL: 5.0	OTHER: 1.6

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In vitro systems are being used to characterize the mechanism by which cyclic nucleotides alter cell function and to explore the mechanism of action of agents whose effect on cell function is mediated by cellular accumulation of cyclic nucleotides.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 53,500-09 DDB

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)
Studies of the Transport of Organic Anions by the Liver

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	E. A. Jones	Chief, Liver Diseases Section	DDB:NIADDK
Other:	J. G. Waggoner	Chemist	DDB:NIADDK

COOPERATING UNITS (if any)

LAB/BRANCH
Digestive Diseases Branch

SECTION
Liver Diseases Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	2.5	0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS XX☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Saturable binding of sulfobromophthalein (BSP) to hepatic canalicular and plasma membranes has been demonstrated. Furthermore, proteins with high affinities for bilirubin and BSP have been isolated from hepatic cell surface membrane fractions suggesting that carrier-mediated transport mechanisms may be of importance in the hepatic uptake and excretion of these organic anions. In studies of bile salt transport it has been shown that microfilaments play a role in hepatic active transport systems. Bilirubin kinetics have been studied before and after splenectomy in 14 patients with hereditary spherocytosis. In 7 hepatic clearance of bilirubin was normal before splenectomy and was unaltered after splenectomy. In the remaining 7 hepatic clearance of bilirubin was subnormal before splenectomy and was increased after splenectomy; in this group hepatic UDP glucuronyl transferase activity was reduced. These findings suggest the existence of a latent state of Gilbert's syndrome which can be unmasked by hemolytic stress.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AM 53,501-09 DDB

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)
Studies of the Pathogenesis of Acute Hepatic Coma and the Use of Artificial Hepatic Support Devices for its Treatment

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	E. A. Jones	Chief, Liver Diseases Sec.	DDB:NIADDK
Other:	S. C. Pappas	Visiting Associate	DDB:NIADDK
	J. G. Waggoner	Chemist	DDB:NIADDK
	P. J. Munson	Investigator	RR:NICHHD
	P. Ferenci	Guest Worker	DDB:NIADDK
	P. Skolnick	Pharmacologist	LBC:NIADDK
	J. T. Fenstermacher	Chief, Membrane Transport Sec.	LCP:NCI

COOPERATING UNITS (if any)

RR:NICHHD
CP:NCI

LAB/BRANCH
Digestive Diseases Branch

SECTION
Liver Diseases Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	1.5	.5

CHECK APPROPRIATE BOX(ES)

X (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The abnormal pattern of visual evoked potentials in rabbits with hepatic encephalopathy (HE) due to fulminant hepatic failure (FHF) resembles that induced by barbiturates and benzodiazepines. As these drugs induce neural inhibition by interacting with receptors for the inhibitory neurotransmitter γ-aminobutyric acid (GABA) on postsynaptic neural membranes, this finding implies that neuronal activity in HE is similar to that induced by GABA. Outside the CNS the main source of GABA is gut bacteria and the main site of its catabolism is the liver. When FHF was induced in rabbits the onset of HE was preceded by a marked increase in plasma GABA levels and by an increase in the permeability of the blood brain barrier (BBB). FHF was associated with a twofold increase in the number of GABA receptors on postsynaptic membranes. These findings suggest that as the liver fails: 1) impaired hepatic metabolism of GABA leads to an increase of gut-derived GABA in plasma, 2) plasma GABA gains access to the brain through a permeable BBB and induces neural inhibition and 3) up-regulation of GABA receptors may increase the sensitivity of the brain to GABA. The status of receptors for other neurotransmitters and the composition of synaptic plasma membranes in HE are currently being studied.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 53,503-08 DDB

PERIOD COVERED

October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Immunologic Studies in Primary Biliary Cirrhosis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	E. A. Jones	Chief, Liver Diseases Section	DDB:NIADDK
Other:	S. P. James	Expert	MET:NCI
	W. Strober	Chief, Immunophysiology Section	MET:NCI
	C. O. Elson	Expert	MET:NCI
	G. Y. Minuk	Medical Staff Associate	DDB:NIADDK

COOPERATING UNITS (if any)

Metabolism Branch, NCI

LAB/BRANCH

Digestive Diseases Branch

SECTION

Liver Diseases Section

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.75	.5	.25

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Abnormal immune mechanisms are being studied in patients with primary biliary cirrhosis (PBC). T cell mediated help and suppression of pokeweed mitogen-induced immunoglobulin synthesis by B cells has been studied using radioimmunoassays to measure IgG and IgM synthesized by cultures of appropriate mixtures of lymphocyte subpopulations in vitro. The ability of T cells to proliferate when cultured with either autologous or allogeneic irradiated B cells (mixed lymphocyte reactions) or with hapten-modified autologous irradiated B cells has been assessed. Results of these studies include the demonstration in PBC of (i) diminished capacity of T cells to inhibit immunoglobulin synthesis in vitro; (ii) a deficiency of the autologous but not the allogeneic mixed lymphocyte reaction and (iii) a deficiency in the primary but not the secondary proliferative response of T cells to hapten-modified cells. These findings suggest that in PBC there is a fundamental defect in the interaction of autoreactive T cells and surface antigens on autologous non-T cells which leads to diminished activation of suppressor T cells and hence predisoposes to a state of immune hyperresponsiveness.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 53,504-08 DDB

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)
Studies of Copper Metabolism in Man Using ^{67}Cu and ^{64}Cu

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	E. A. Jones	Chief, Liver Diseases Section	DDB:NIADDK
Other:	R. Aamodt	Senior Radiobiologist	NM:CC
	W. Rumble	Biologist/Radiobiologist	NM:CC
	R. Shrager	Mathematician	LSMM:CR

COOPERATING UNITS (if any)
Nuclear Medicine Department, CC
Laboratory of Statistical and Mathematical Methodology, CR

LAB/BRANCH
Digestive Diseases Branch

SECTION
Liver Diseases Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0	PROFESSIONAL: 0	OTHER: 0

CHECK APPROPRIATE BOX(ES)
☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project has been terminated.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 53,505-07 DDB

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Studies of the Metabolism of Alpha-1-Antitrypsin and of Protease Inhibitor Phenotypes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	E. A. Jones	Chief, Liver Diseases Section	DDB:NIADDK
Other:	J. Vergalla	Chemist	DDB:NIADDK

COOPERATING UNITS (if any)

Pulmonary Branch, NHLBI

LAB/BRANCH
Digestive Diseases Branch

SECTION
Liver Diseases Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	.25	.25

CHECK APPROPRIATE BOX(ES)

XX ☒ (a) HUMAN SUBJECTS   X ☒ (b) HUMAN TISSUES   ☐ (c) NEITHER

☐ (a1) MINORS   ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Isolated preparations of α-1-antitrypsin (α1AT), trace-labeled with radioiodine, have been used to obtain data on the turnover of α1AT in normal subjects and patients with α1AT deficiency who have either pulmonary emphysema or chronic hepatocellular disease. The protease inhibitor (Pi) phenotypes of subjects donating plasma for isolation of α1AT and subjects undergoing α1AT turnover studies have been determined. The fractional catabolic rates of M α1AT from normal subjects (phenotype PiMM) and Z α1AT from patients with α1AT deficiency (phenotype PiZZ) are similar in both normal subjects and patients with α1AT deficiency. These findings imply that the low serum α1AT concentration in α1AT deficiency is due to impaired hepatic release of α1AT and that the differences in molecular structure between the Z and M proteins have little effect on the catabolism of α1AT. In common with other glycoproteins, desialylated (i.e. neuraminidase-treated) α1AT is rapidly cleared from plasma by the liver. Pi phenotypes have been determined in populations of normal subjects and patients with rheumatoid arthritis, systemic lupus erythematosus, Sjogren's syndrome and hepatocellular carcinoma.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 53,506-06 DDB

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Studies of the Properties of Desialylated Human Lymphocytes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P. I.:	E. A. Jones	Chief, Liver Diseases Section	DDB:NIADDK
Other:	C. J. Steer	Expert	LBM:NIADDK
	S. P. James	Expert	MET:NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Digestive Diseases Branch

SECTION
Liver Diseases Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0	PROFESSIONAL: 0	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is currently inactive.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 53,508-05 DDB

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Studies of Hepatic Receptors for Modified Glycoproteins

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	E. A. Jones	Chief, Liver Diseases Section	DDB:NIADDK
Other:	G. Ashwell	Chief, Lab. Biochem. and Metab.	LBM:NIADDK
	J. Vergalla	Chemist	DDB:NIADDK

COOPERATING UNITS (if any)

Laboratory of Biochemistry and Metabolism

LAB/BRANCH
Digestive Diseases Branch

SECTION
Liver Diseases Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	.25	.25

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The cellular location and carbohydrate specificies of a glycoprotein recognition system on rat hepatic sinusoidal cells have been determined. Purified preparatons of endothelial, Kupffer and parenchymal cells have been prepared in situ by collagenase liver perfusion, centrifugation on Percoll gradients and centrifugal elutriation. ^{125}I-labeled agalactoorosomucoid (AGOR), an N-acetylglucosamine-terminated glycoprotein, is selectively and specifically taken up in vitro by endothelial cells. Glucose and a glucose-albumin conjugate competitively inhibited this uptake process over a wide range of concentrations. Uptake by cells from fasted rats was enhanced, but uptake by cells from fasted or fed diabetic rats was normal. The in vivo hepatic uptake and catabolism of ^{125}I-AGOR were slower in diabetic than normal rats. These findings indicate that 1) the hepatic receptors which recognize N-acetylglucosamine/mannose terminated glycoproteins are located on endothelial cells, 2) these receptors are glucose sensitive, 3) fasting increases the number of these receptors and 4) diabetes mellitus abolishes this effect of fasting and impairs the function of this receptor in vivo. The results of this study suggest a mechanism for abnormal glycoprotein metabolism in diabetes mellitus.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 53,509-04 DDB

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Studies of patients who are chronic carriers of hepatitis B surface antigen (HBsAg)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	J. H. Hoofnagle	Senior Investigator	DDB:NIADDK
Other:	E. A. Jones	Chief, Liver Diseases Section	DDB:NIADDK
	G. Y. Minuk	Clinical Associate	DDB:NIADDK
	R. G. Hanson	Clinical Associate	DDB:NIADDK
	S. C. Pappas	Clinical Associate	DDB:NIAMDD
	R. H. Purcell	Chief, Hepatitis Section	LID:NIAID
	J. Gerin	Head, Molec. Virol. & Immunol. Div.	Georgetown University
	L. B. Seeff	Chief, Gastroenterology	VA Medical Center, Washington, D.C.
	S. E. Straus	Senior Investigator	LCI:NIAID
	S. P. James	Expert	LIP:NCI

COOPERATING UNITS (if any)
LID and LCI, NIAID
Georgetown University School of Medicine; VA Hospital, Washington, D.C.

LAB/BRANCH
Digestive Diseases Branch

SECTION
Liver Diseases Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	1.5	0.5

CHECK APPROPRIATE BOX(ES)
☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Patients with chronic type B hepatitis are being studied. Approximately 150 patients are followed regularly in the outpatient clinic; serologic markers of hepatitis B virus (HBV) are determined frequently in serum. Selected individuals are entered into controlled therapeutic trials of either anti-viral or immunosuppressive drug therapy. In a randomized, controlled study of adenine arabinoside 5' monophosphate (Ara-AMP), a one-month course of drug did not lead to a significantly higher rate of clinical improvement (30%) than occurred in a control group receiving no therapy (20%). Further studies of a single, high-dose course of prednisolone, multiple courses of Ara-AMP and every other day therapy with recombinant DNA human leukocyte A interferon are currently in progress. The pathogenesis of chronic type B hepatitis is being investigated using in vitro assays of immunologic function. A specific defect in anti-HBs synthesis by chronic HBsAg carriers has been identified. The etiology of this defect is being pursued and appears to be a consequence of the large amounts of HBsAg in the circulation of most carriers inhibiting the proliferation of clones of anti-HBs synthesizing lymphocytes.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 53,510-03 DDB

PERIOD COVERED

October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Study of the Natural History of Chronic Non-A, Non-B Hepatitis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	J. H. Hoofnagle	Senior Investigator	DDB:NIADDK
Other:	H. J. Alter	Chief, Immunology Section	BB:CC
	E. A. Jones	Chief, Liver Diseases Section	DDB:NIADDK
	S. C. Pappas	Visiting Associate	DDB:NIADDK
	R. G. Hanson	Visiting Associate	DDB:NIADDK
	R. H. Purcell	Chief, Hepatitis Section	LID:NIAID
	G. L. Davis	Medical Staff Fellow	DDB:NIADDK
	S. M. Feinstone	Senior Investigator	LID:NIAID
	K. Ishak	Chief, Hepatic Pathology Branch	A.F.I.P.
	G. Y. Minuk	Visiting Associate	DDB:NIADDK

COOPERATING UNITS (if any)

NIH Blood Bank
Hepatitis Section, LID:NIAID
Armed Forces Institute of Pathology

LAB/BRANCH

Digestive Diseases Branch

SECTION

Liver Diseases Section

INSTITUTE AND LOCATION

NIADDK, Bethesda, Maryland 20205

TOTAL MANYEARS: .75	PROFESSIONAL: .75	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Patients with well-documented chronic non-A, non-B (non-lupoid) hepatitis are being evaluated to determine the natural history of this disease and to obtain tissues and reagents to search for non-A, non-B agents. Twenty-eight patients have been admitted to this study. These patients have undergone thorough evaluation including a liver biopsy. They have had the disease for periods ranging from 1 to 10 years. Six patients have a mild degree of malaise, the remaining patients are asymptomatic. Liver biopsies have revealed chronic persistent hepatitis in six, chronic active hepatitis in twenty, and alcoholic liver disease in two. Six patients have developed cirrhosis. These studies have provided a framework for trials of immunosuppressive and antiviral therapy. A pilot study of Acyclovir therapy has been initiated.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do **NOT** use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 53,511-03 DDB

PERIOD COVERED

October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Controlled Trial of Chlorambucil Therapy in Primary Biliary Cirrhosis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.	E. A. Jones	Chief, Liver Diseases Section	DDB:NIADDK
Other:	J. H. Hoofnagle	Senior Investigator	DDB:NIADDK
	G. Y. Minuk	Clinical Associate	DDB:NIADDK
	S. C. Pappas	Clinical Associate	DDB:NIADDK
	R. G. Hanson	Clinical Associate	DDB:NIADDK
	G. L. Davis	Clinical Associate	DDB:NIADDK

COOPERATING UNITS (if any)

LAB/BRANCH

Digestive Diseases Branch

SECTION

Liver Diseases Section

INSTITUTE AND LOCATION

NIADDK, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	1.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) X HUMAN SUBJECTS ☐ (b) X HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Symptomatic primary biliary cirrhosis (PBC) appears to be a uniformly fatal disease characterized by slowly progressive intrahepatic cholestasis due to a chronic non-suppurative destructive cholangitis involving the septal and larger interlobular bile ducts. Trials of corticosteroids and azathioprine have not shown that these agents effectively inhibit the progression of the disease or increase survival. Because certain chronic inflammatory diseases appear to respond favorably to alkylating agents but not to azathioprine, a controlled randomized trial of an alkylating agent for the treatment of PBC has been initiated. The alkylating agent chosen for the trial was chlorambucil. Twenty-four patients (23 women, 1 man; ages 34-63) have been admitted this trial: 13 were randomized to the treatment and 11 to the control group. Twelve patients have been followed for 2 years. Among these 12 (7 treated, 5 controls) 3 of the untreated patients have developed bleeding from esophageal varices and 2 have had significant deepening of jaundice (bilirubin rising J 10 mg% since admission into the trial). None of the treated patients have developed variceal bleeding or deepening of jaundice.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 53,512-03 DDB

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Studies of the Uptake of Lipoproteins by Hepatic Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	E. A. Jones	Chief, Liver Diseases Section	DDB:NIADDK
Other:	J. Vergalla	Chemist	DDB:NIADDK
	E. Schaefer	Senior Investigator	MD:IR:NHLBI
	B. Brewer	Chief, Mol. Dis. Br.	MD:IR:NHLBI

COOPERATING UNITS (if any)
Molecular Diseases Branch, NHLBI

LAB/BRANCH
Digestive Diseases Branch

SECTION
Liver Diseases Section

INSTITUTE AND LOCATION
NIADDK, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	0.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Studies are designed to determine which of the major classes of hepatic cells are responsible for the uptake of different lipoproteins by the liver and to define which apoproteins mediate the uptake of lipoproteins by the different cell types. Rat endothelial, Kupffer and parenchymal cells are isolated by collagenase liver perfusion, centrifugation on Percoll gradients and centrifugal elutriation. Apoproteins isolated from normal subjects and patients with Tangier disease have been radioiodinated and reassociated with lipoprotein particles from normal subjects and patients with Tangier disease. Measurements of the uptake of these labeled particles by hepatic cells in vitro indictate that the increased reticuloendothelial (RE) cell uptake of cholesterol in Tangier disease is probably mediated by enhanced uptake of Tangier apoprotein A-I-containing cholesterol-rich lipoproteins by RE cells and that the rapid clearance of lipoproteins from the plasma in Tangier disease can be accounted for by the uptake of Tangier apoprotein A-I-containing lipoproteins by hepatocytes. Thus, in Tangier disease a structurally abnormal apoprotein A-I appears to be responsible for the mediation of hypercatabolism of lipoproteins and the abnormal accumulation of lipoproteins in RE cells.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 53,513-02 DDB

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)
Studies of the Role of Chemotaxis in the Pathogenesis of Hepatic Fibrosis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.	W. G. Cooksley	Visiting Scientist	DDB:NIADDK
Other:	G. Grotendorst	Post-doctoral Fellow	LDBA:NIDR
	C. McIvor	Guest Worker	DDB:NIADDK
	J. Vergalla	Chemist	DDB:NIADDK
	E. A. Jones	Chief, Liver Diseases Section	DDB:NIADDK

COOPERATING UNITS (if any)

Laboratory of Developmental Biology and Anomalies, NIDR

LAB/BRANCH
Digestive Diseases Branch

SECTION
Liver Diseases Section

INSTITUTE AND LOCATION
NIADDK, NIH Bethesda, Maryland 20205

TOTAL MANYEARS: .75	PROFESSIONAL: 0.5	OTHER: .25

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The fibrosis in the liver which can occur pari passu with chronic hepatocellular injury may arise as a result of increased collagen synthesis by connective tissue-derived cells which have migrated to the liver in response to the release of chemotactic factors. The nature of these putative chemotactic factors, their cell of origin and the sequence of events which leads to their release and biological activity are unknown. Using an in vitro assay to measure the response of smooth muscle cells (SMC) to chemotactic factors it has been shown that normal rat Kupffer cells (but not hepatocytes or hepatic endothelial cells) elaborate chemoattractants for SMC. It has also been shown that monocytes from chronic type B hepatitis blood but not from normal blood synthesize chemoattractants for SMC. These findings are consistent with the hypothesis that hepatic fibrogenesis can arise as a result of collagen synthesis by modulated SMC after their migration to the liver in response to the release of chemoattractants.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 54016-07 DD^B

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Lipoprotein Composition in Pima Indians

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B.V. Howard	Associate Chief	PCRS, NIADDK
OTHER:	P.H. Bennett	Chief	EFSB, NIADDK
	W.C. Knowler	Chief	SFSS, NIADDK
	D.J. Pettitt	Assistant Chief	SFSS, NIADDK

COOPERATING UNITS (if any) E. Nikkila, M. Taskinen, Univ. of Helsinki, Finland; R.H. Unger, Univ. of Texas Southwestern Medical School, Dallas; J. Fuller and M. Mattock, Guys Hospital, London, England

LAB/BRANCH
Digestive Diseases Branch

SECTION
Phoenix Clinical Research Section

INSTITUTE AND LOCATION
NIADDK, NIH, Phoenix, Arizona 85016

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.3	.5	.8

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Both low density lipoproteins and very low density lipoproteins have been implicated as positive risk factors in the genesis of atherosclerosis, whereas high density lipoprotein concentration is an independent negative risk factor. Both diabetes and obesity are associated with changes in plasma lipoproteins and these changes may be related to the increased prevalence of cardiovascular disease. We are studying lipoprotein composition in obese and diabetic Pima Indians. Cholesterol and triglycerides are being measured in VLDL, LDL, and HDL, as well as in HDL subclasses isolated by ultracentrifugation. These parameters are being compared in groups of normal and diabetic Indians with and without coronary artery disease, and the influence of therapy is being assessed. Apoprotein composition of specific lipoproteins will be measured. From this study we will attempt to determine if diabetes and obesity are associated with changes in serum lipoproteins and to delineate the relation between lipoprotein levels and the pathogenesis of ischemic heart disease.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 54017-06 DDB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Effects of Weight-reduction on Glucose Tolerance and Gluco-regulatory Hormones

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. Bogardus	Acting Chief	PCRS, NIADDK
OTHER:	M. Nagulesparan	Guest Worker	PCRS, NIADDK
	B. Vasquez	Staff Fellow	PCRS, NIADDK
	M.A. Verso	Staff Physician	PCRS, NIADDK

COOPERATING UNITS (if any)
R.H. Unger, University of Texas Health Science Center, Dallas
A.H. Rubenstein, University of Chicago, School of Medicine, Chicago

LAB/BRANCH
Digestive Diseases Branch

SECTION
Phoenix Clinical Research Section

INSTITUTE AND LOCATION
NIADDK, NIH, Phoenix, Arizona 85016

TOTAL MANYEARS: 1.0	PROFESSIONAL: .2	OTHER: .8

CHECK APPROPRIATE BOX(ES)
☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER
☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Six patients with recent-onset, noninsulin dependent diabetes underwent assessment of glucose tolerance, insulin secretory capacity, and insulin-induced glucose disposal before and after 6-10 weeks of intensive aerobic training while maintaining body weight. Fasting plasma glucose declined in every subject (average= -33mg/dl), and oral glucose tolerance (3 h integrated plasma glucose) improved in five of the six (average=-74mg·3h/dl) after training. These improvements were associated with one or more of the following: (1) an increase in the early (30 minute) plasma insulin response to oral glucose (average=39μU/ml), (2) an increase in the 3h integrated plasma insulin response to oral glucose (average =+33μU·3h/ml), (3) an increase in the insulin-induced glucose disposal rate during the euglycemic glucose clamp (average=+0.6mg/kg/min=+27%), and/or (4) a decrease in the fasting plasma insulin level (average=-11μU/ml). Of these improvements, only the increase in early insulin response was seen in every subject. Our results show that regular endurance exercise is effective in improving glucose homeostasis and may serve as an adjunct to other modes of treatment in recent-onset noninsulin dependent diabetics.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 54018-06 DDB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Characterization of Metabolic and Morphologic Abnormalities in Diabetes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. Bogardus	Acting Chief	PCRS, NIADDK
OTHER:	M. Nagulesparan	Guest Worker	PCRS, NIADDK
	J. Roth	Chief	DB, NIADDK
	B.V. Howard	Associate Chief	PCRS, NIADDK
	D.M. Mott	Research Biochemist	PCRS, NIADDK
	I. Klimes	Guest Worker	PCRS, NIADDK
	B. Vasquez	Staff Fellow	PCRS, NIADDK

COOPERATING UNITS (if any)
R.H. Unger, Human Diabetes Study Center, University of Texas Health Science Center, Dallas, TX; R. Andres, NIA, Baltimore, MD; D. Elahi, NIA, Baltimore, MD; A.H. Rubenstein, Univ. of Chicago

LAB/BRANCH
Digestive Diseases Branch

SECTION
Phoenix Clinical Research Section

INSTITUTE AND LOCATION
NIADDK, NIH, Phoenix, Arizona 85016

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	.4	.8

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Age, sex and weight matched groups of genetically normal, prediabetic and diabetic Pima Indians and normal Caucasians were examined by means of oral and intravenous glucose tolerance tests, arginine infusions, in vivo insulin resistance measurements, glucose clamp technique and circulatory mononuclear cell insulin receptor assays, and urinary protein profiles to determine whether abnormalities of insulin, glucagon secretion or other measurements are characteristic of the prediabetic, diabetic, obese and nonobese states.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 54019-06 DDB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Cellular Metabolism in Diabetic and Nondiabetic Subjects

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B.V. Howard	Associate Chief	PCRS, NIADDK
OTHER:	B. Vasquez	Staff Fellow	PCRS, NIADDK

COOPERATING UNITS (if any) J. Larner, Dept. of Pharmacology, Univ. of Virginia School of Medicine; Human Diabetes Study Center, Univ. of Texas Southwestern Medical School, Dallas; H. Hidaka, Univ. of Osaka, School of Medicine, Japan; F. Ishibashi, Hiroshima Univ., School of Medicine, Japan

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Digestive Diseases Branch

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Phoenix Clinical Research Section

INSTITUTE AND LOCATION
NIADDK, NIH, Phoenix, Arizona 85016

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	.2	.8

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Elevated insulin levels and insulin resistance in Pima Indian diabetics and nondiabetics suggest that cellular changes external to the panceras may be important in the pathogenesis of obesity and diabetes mellitus. The aim of this project is to study several aspects of insulin function in cells obtained from normal and diabetic Pima Indians, in order to determine if diabetes mellitus is expressed in genetic differences in insulin action at the cellular level. Fibroblast cultures have been established from normal and diabetic Pima Indians. Cellular metabolism and transport functions are being studied in the presence and absence of insulin, in order to define cell insulin action in this system, to determine if cellular abnormalities in insulin response occur in individuals with diabetes and to investigate factors which may induce cell insulin resistance. Stimulation of rapid metabolic effects by insulin and the insulin-like growth factor, MSA, are being compared to investigate the functional relationships between receptors for these peptide hormones in fibroblasts.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 54020-05 DDB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Regulation of Insulin Receptors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D.M. Mott	Research Biochemist	PCRS, NIADDK
OTHER:	B.V. Howard	Associate Chief	PCRS, NIADDK
	M. Nagulesparan	Guest Worker	PCRS, NIADDK
	B. Vasquez	Staff Fellow	PCRS, NIADDK

COOPERATING UNITS (if any)

Human Diabetes Study Center, University of Texas Health Science Center

LAB/BRANCH
Digestive Diseases Branch

SECTION
Phoenix Clinical Research Section

INSTITUTE AND LOCATION
NIADDK, NIH, Phoenix, Arizona 85016

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.6	.2	.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Regulation of insulin receptors is being studied in vivo using circulating mononuclear cells and in vitro using cultured diploid fibroblasts. Investigation of monocytes and fibroblasts suggest that cells from obese subjects have more receptors, reduced insulin affinity, increased insulin degradation and decreased down regulation. Insulin fibroblasts and from human erythrocytes. Characterization of insulin binding to solubilized insulin receptor preparations resulted in binding affinity, pH optimum and analog specificity data similar to those for the intact cells. The binding of an insulin-like growth factor, Multiplication Stimulating Activity (MSA), as well as insulin were measured in suspended human fibroblasts and in solubilized receptor preparations. Neither Sephadex G-200 chromatography, wheat germ lectin chromatography nor nondenaturing polyacrylamide gel electrophoresis (with 0.1% Triton) separated the two receptors, although characterization of the specificity of binding clearly predicts two separate binding sites.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ZM 54021-05 DDB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Hemoglobin A1 Levels in Diabetes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J.E. Foley	Senior Staff Fellow	PCRS, NIADDK
OTHER:	W.C. Knowler	Chief	SFSS, NIADDK

COOPERATING UNITS (if any)

LAB/BRANCH
Digestive Diseases Branch

SECTION
Phoenix Clinical Research Section

INSTITUTE AND LOCATION
NIADDK, NIH, Phoenix, Arizona 85016

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.6	.2	.4

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The quantitative determination of glycosylated hemoglobins reflects a subject's average blood glucose concentration over a long period. It is not affected by short term fluctuations in blood sugar and therefore, provides a precise reflection of blood glucose control. The measurement of glycosylated hemoglobin in combination with other glucose tolerance tests can provide a more accurate estimate of glucose intolerance in diabetic subjects.

Previous work has shown that increased glycosylated hemoglobin levels in diabetic Pima Indians versus nondiabetic Pima Indians. However exact quantitation of the correlation has not been successful. We are currently testing a new electrophoretic method of separating glycosylated hemoglobin (Corning Medical and Scientific, Medford, MA) from nonglycosylated hemoglobin.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AM 54023-05 DDB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Lipoprotein Metabolism in Pima Indians

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B.V. Howard	Associate Chief	PCRS, NIADDK
OTHER:	L. Zech	Staff Physician	IRMD, NHLBI
	G. Egusa	Visiting Fellow	PCRS, NIADDK

COOPERATING UNITS (if any) S.M. Grundy and W. Bettz, Dept. of Medicine, Univ. of Texas Southwestern Medical School, Dallas, TX; Marja Riita Taskinen, Dept. of Medicine, Univ. of Helsinki, Finland

LAB/BRANCH
Digestive Diseases Branch

SECTION
Phoenix Clinical Research Section

INSTITUTE AND LOCATION
NIADDK, NIH, Phoenix, Arizona 85016

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.6	.8	1.8

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Pima Indians have a high prevalence of obesity, diabetes mellitus and hyperinsulinemia, but they have low plasma cholesterol levels, reduced LDL synthesis, and decreased incidence of cardiovascular disease. Lipoproteiin metabolism in Pima Indians will be investigated in order to further investigate lipoprotein metabolism in this population. Synthetic and catabolic rates for VLDL will be compared in groups having different insulin levels using tritiated glycerol as a precursor. VLDL metabolism will be studied in a group of diabetics to determine the influence of hyperglycemia on VLDL metabolism in this population. Conversion of VLDL to LDL will be studied in nondiabetics and diabetics using ^{131}I VLDL and ^{125}I LDL simultaneously in order to elucidate the mechanism of maintenance of low LDL. Postheparin, muscle, and adipose tissue lipoprotein lipase levels will be measured. The results of this study should yield data on the control of lipoprotein metabolism in this population and explore the relation between these lipoproteins, diabetes and cardiovascular disease.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 54024-05 DD B

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
In Vitro Insulin Resistance in Pima Indians

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. Foley	Senior Staff Fellow	PCRS, NIADDK
OTHER:	A. Kashiwagi	Fogarty Fellow	PCRS, NIADDK
	C. Bogardus	Chief	PCRS, NIADDK
	M. Antonia Verso	Staff Physician	PCRS, NIADDK
	B.V. Howard	Associate Chief	PCRS, NIADDK
	S. Cushman	Associate Chief	CMOS, NIADDK
	L. Salans	Director	NIADDK
	B. Vasquez	Staff Fellow	PCRS, NIADDK
	J. Andrews	Guest Worker	PCRS, NIADDK

COOPERATING UNITS (if any)
Gerald Reaven, Stanford University and VA Hospital, Palo Alto, CA

LAB/BRANCH
Digestive Diseases Branch

SECTION
Phoenix Clinical Research Section

INSTITUTE AND LOCATION
NIADDK, NIH, Phoenix, Arizona 85016

TOTAL MANYEARS: 5.2	PROFESSIONAL: 1.7	OTHER: 3.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We have developed methods to measure insulin binding glucose transport, glucose metabolism and lipolysis in adipocytes isolated from human sucutaneous abdominal tissue. We have found in Pima Indians that measurements of maximum insulin-stimulated glucose transport and metabolism are decresed, and measurements of basal lipolysis are increased in obese subjects with NIDDM compared with measurements in obese glucose tolerance subjects. These alterations are correlated with fasting and two hour postload plasma glucose concentrations suggesting that this in vitro insulin resistance is a reflecting in vivo insulin resistance. Furthermore, we are able to reverse this in vitro insulin resistance concurrent with reversal of in vivo insulin resistance with 3 weeks of insulin therapy. We will follow-up on these findings in a longitudinal study of adult Pima Indians with high and low probabilities of developing diabetes in the next 5 years. Our studies of insulin action in human and rat adipocytes and adipocyte membranes have suggested a role for catecholamines and cyclic AMPin controlling the sensitivity of glucose transport to insulin and a role for substrate control of the glucose transport system. The latter is consistent with the correlation between in vivo glucose concentrations and the in vitro glucose transport rates.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 54025-04 DDB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

$(Na^+ + K^+)$ ATPase Activity in Obese Subjects

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D.M. Mott	Research Biochemist	PCRS, NIADDK
OTHER:	B.V. Howard	Associate Chief	PCRS, NIADDK
	C. Bogardus	Acting Chief	PCRS, NIADDK

COOPERATING UNITS (if any)

LAB/BRANCH
Digestive Diseases Branch

SECTION
Phoenix Clinical Research Section

INSTITUTE AND LOCATION
NIADDK, NIH, Phoenix, Arizona 85016

TOTAL MANYEARS: 2.5	PROFESSIONAL: 1.1	OTHER: 1.4

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The level of Na-pump activity has been implicated in mechanisms which could contribute to human obesity. Na, K-ATPase activity is reduced in intact red cells and isolated erythrocyte membranes from obese Pima Indian subjects. Isolated adipocytes had diminished insulin stimulation of Na,K-ATPase activity in obese compared to lean subjects. In addition, caloric restriction in vivo which has been shown to reduce metabolic rate, resulted in a reduced basal and maximal insulin stimulated Na,K-ATPase activity in isolated fat cells. No differences were observed, however, in the basal or insulin stimulated Na-pump activity for cultured diploid fibroblasts. Thus, diminished KaKAA may not be an inherent defect, but may be a manifestation of the insulin resistance state which contributes to the development of obesity via decreased cellular thermogenesis.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 54027-03 DDB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Effect of HGH Therapy on Somatostatin and Pancreatic PP and Lipoprotein Levels in GH Deficient Subjects

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. Bogardus	Acting Chief	PCRS, NIADDK
OTHER:	B.V. Howard	Associate Chief	PCRS, NIADDK
	M. Nagulesparan	Guest Worker	PCRS, NIADDK

COOPERATING UNITS (if any) R. Gingerich, Dept. of Pediatrics, Washington Univ. School of Medicine, St. Louis, MO; R.H. Unger, Human Diabetes Study Center, Univ. of Texas, Southwestern Medical School, Dallas, TX.

LAB/BRANCH
Digestive Diseases Branch

SECTION
Phoenix Clinical Research Section

INSTITUTE AND LOCATION
NIADDK, NIH, Phoenix, Arizona 85016

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Growth hormone deficient subjects who met the criteria for growth hormone therapy from the National Pituitary Agency are being selected for study. Serum lipoprotein composition is being measured before, during and after growth hormone therapy. Similarly, serum somatostatin and pancreatic polypeptide levels following a test meal are being measured in these same subjects to determine the effects of growth hormone therapy on these parameters. At this time only one patient has been recruited for this study. After one month of growth hormone therapy, slight decreases in fasting plasma triglyceride and insulin levels at 30 and 60 minutes after the test meal were noted. Additional patients need to be recruited in order to continue this study.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 54028-03 DDB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Determination of Gastric Acid Secretion in Pima Indians

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: C. Bogardus	Acting Chief	PCRS, NIADDK
OTHER: M. Nagulesparan	Guest Worker	PCRS, NIADDK
H. Sasaki	Guest Worker	PCRS, NIADDK

COOPERATING UNITS (if any) R.H. Unger, Human Diabetes Study Center, Phoenix, AZ: Eugene Straus, V.A. Hospital, Bronx, NY; Roslyn Yalow, V.A. Hospital, Bronx, NY; I.L. MacGregor, V.A. Med. Center, Tucson, AZ; A. Dubois, Uniform Services Hosp. Bethesda, MD

LAB/BRANCH
Digestive Diseases Branch

SECTION
Phoenix Clinical Research Section

INSTITUTE AND LOCATION
NIADDK, NIH, Phoenix, Arizona 85016

TOTAL MANYEARS: 3.6	PROFESSIONAL: 1.3	OTHER: 2.3

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The findings of low acid production, hypergastrinemia, and slow emptying of the stomach suggests that hypochlorhydria may be an important reason for the rarity of duodenal ulcer in Pima Indians. There is no evidence that liquid gastric emptying rates are altered in obesity. The hyperinsulinemia of obesity does not appear to be mediated by the gastric emptying rate or by circulating plasma SLI levels. Hypergastrinemia occurs in Type II diabetes, apparently as a result of autonomic dysfunction with slow gastric emptying.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 54029-02 DDB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Nutrition-Induced Alterations in Metabolism

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. Bogardus	Acting Chief	PCRS, NIADDK
OTHER:	B. Daniels	Staff Fellow	PCRS, NIADDK
	M. Nagulesparan	Guest Worker	PCRS, NIADDK
	B.V. Howard	Associate Chief	PCRS, NIADDK
	R.F. Goldman	Director	U.S.A. Ergonomics Lab
	M. Lawson	Chief, Nuclear Medicine	Good Samaritan Hosp.

COOPERATING UNITS (if any)
E. Danforth, Jr., E.A.H. Sims, E. Horton, Dept. of Medicine, University of Vermont, Burlington, VT; L. Landsburg, Dept. of Medicine, Harvard University

LAB/BRANCH
Digestive Diseases Branch

SECTION
Phoenix Clinical Research Section

INSTITUTE AND LOCATION
NIADDK, NIH, Phoenix, Arizona 85016

TOTAL MANYEARS: 5.5	PROFESSIONAL: 1.7	OTHER: 3.8

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

It has been proposed that the high incidence of obesity in the Pima Indians may be due to genetic selection for a "thrifty gene", a gene that would predispose the Pimas to store energy very efficiently. These efficient individuals would presumably be more capable of surviving famine than lean, less efficient individuals. In order to determine whether or not such a genetic difference exists, resting metabolic rates and responses to three thermogenic stimuli (food, norepinephrine and exercise) were measured. Since overeating obviously precedes obesity, the aforementioned parameters were measured during weight maintenance as well as during overfeeding; certain individuals were also studied during underfeeding. Thyroid kinetics studies were also performed on a group of obese Pima volunteers, since these hormones have been shown to be affected by the nutritional state of an individual. The results from this research are being compared to those obtained from lean and obese Caucasians involved in studies performed in Vermont.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 54030-02 DDB

PERIOD COVERED
October 1, 1981 to September 31, 1982

TITLE OF PROJECT (80 characters or less)

Radioimmunoassay of Polypeptide Hormones

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. Vasquez	Staff Fellow	PCRS, NIADDK
OTHER:	I. Klimes	Guest Worker	PCRS, NIADDK
	W.C. Knowler	Chief	SFSS, NIADDK

COOPERATING UNITS (if any)
R.H. Unger, Human Diabetes Study Center, University of Texas Health Science Center

LAB/BRANCH
Digestive Diseases Branch

SECTION
Phoenix Clinical Research Section

INSTITUTE AND LOCATION
NIADDK, NIH, Phoenix, Arizona 85016

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	.3	.8

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The radioimmunoassay (RIA) laboratory supports epidemiological (in collaboration with EFSB, NIADDK) and metabolic studies which address the role of islet cell function and insulin resistance in the development and maintenance of obesity and diabetes in the Pima population. RIA's for insulin, C-peptide, glucagon, glucagon-like immunoreactivity (GLI) and somatostatin (SLI) and are performed in conjunction with clinical studies described in reports on projects Z01 AM 69000-17 EFS, Z01 AM AM54031-01 DD, Z01 AM 54029-02 DD, and are central to the investigation of islet function and regulation in obesity and diabetes.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 54031-01 DD B

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

The Effect of One Month's Insulin Therapy on Peripheral Insulin Action and Hepatic Glucose Output in Noninsulin Dependent Diabetic Pima Indians

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. Howard	Associate Chief	PCRS, NIADDK
OTHER:	W.J. Andrews	Guest Worker	PCRS, NIADDK
	M. Nagulesparan	Guest Worker	PCRS, NIADDK
	B. Vasquez	Staff Fellow	PCRS, NIADDK
	G.M. Reaven	Consultant	Stanford VA Hospital
	R.H. Unger	Consultant	Dallas VA Hospital

COOPERATING UNITS (if any)

LAB/BRANCH
Digestive Diseases Branch

SECTION
Phoenix Clinical Research Section

INSTITUTE AND LOCATION
NIADDK, NIH, Phoenix, Arizona 85016

TOTAL MANYEARS: 5.0	PROFESSIONAL: 1.2	OTHER: 3.8

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

After diet stabilization insulin action was measured at plasma insulin levels of 100μU/ml and 250μU/ml using the euglycemic clamp technique, in thirteen obese noninsulin dependent diabetics (NIDDM). Fasting and two hour blood glucose concentrations were then normalized for a period of one month using insulin. After this period insulin action was reassessed similarly in all subjects and then reassessed a further two weeks after therapy withdrawal. The results show that normalization of blood glucose over one month improves insulin action at plasma insulin concentrations of 100μU/ml ($P<0.05$) and 250μU/ml ($P<0.003$). Further this improvement is maintained for at least weeks after therapy withdrawal. Thus it appears that hyperglycemia contributes to the impaired insulin action of NIDDM, and that insulin action can be improved by normalization of blood glucose, without weight loss, in NIDDM.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 54032-01 DDB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Cellular Metabolism of Cultured Human Adipocyte Precursors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. Vasquez	Staff Fellow	PCRS, NIADDK
OTHER:	Antonia Verso	Staff Physician	PCRS, NIADDK
	J. Foley	Senior Staff Fellow	PCRS, NIADDK
	B.V. Howard	Associate Chief	PCRS, NIADDK

COOPERATING UNITS (if any)

LAB/BRANCH
Digestive Diseases Branch

SECTION
Phoenix Clinical Research Section

INSTITUTE AND LOCATION
NIADDK, NIH, Phoenix, Arizona 85016

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.3	.8	1.5

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER
☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Cultured human adipocytes precursors (HAPs) are a promising culture system for investigation of inherent differences in cellular metabolism in obesity and diabetes HAPs were established in culture from the stromal vascular fraction of collagenase digests of abdominal subcutaneous fat. HAPs were stimulated to initiate the differentiation process to mature adipocytes in vitro in the presence of insulin (5 x 10^{-8}m), as assessed by triglyceride deposition. In order to study an acute action of insulin a method for measuring initial rates of glucose transport in confluent monolayers of HAPSwas developed.. The glucose transport system was chosen because insulin stimulation ofthe glucose transporter has been extensively investigated in mature adipocytes. Insulin stimulated glucose transport in HAPs with a mean (n=5) ED50 of $3x10^{-9}$M insulin. Maximally stimulated rate (137.5±10.5nL/sec ng) was twice basal (63.4±9.8nL/sec ng). Mature adipocytes may still reflect the in vivo milieu at the time of biopsy. An acute action of insulin in HAPs has been defined that can now be compared to similar data obtained for mature adipocytes from the same biopsy. Thus, this system may provide a tool for distinguishing between environmentally and genetically determined alterations in cellular metabolism that contribute to the increased insulin resistance associated with obesity and diabetes.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 54033-01 DDB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Pathophysiology of the Adipocyte in Human Obesity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J.E. Foley	Senior Staff Fellow	PCRS, NIADDK
OTHER:	D. Pettitt	Assistant Chief	SFSS, NIADDK
	C. Bogardus	Acting Chief	PCRS, NIADDK
	B. Howard	Associate Chief	PCRS, NIADDK
	B. Vasquez	Staff Fellow	PCRS, NIADDK
	M.A. Verso	Staff Physician	PCRS, NIADDK
	A. Kashiwagi	Fogarty Fellow	PCRS, NIADDK
	P. Bennett	Chief	EFSB, NIADDK
	W.C. Knowler	Chief	SFSS, NIADDK
	S.W. Cushman	Associate Chief	COMS, NIADDK
	L. Salans	Director	NIADDK

COOPERATING UNITS (if any)

Gerald Reaven, Stanford University and VA Hospital, Palo Alto, CA

LAB/BRANCH
Digestive Diseases Branch

SECTION
Phoenix Clinical Research Section

INSTITUTE AND LOCATION
NIADDK, NIH, Phoenix, Arizona 85016

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.0	1.2	2.8

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The Pima Indians are a group of people with a very high incidence of obesity, which is already developing in the children and adolescents of this population. Adipose cell metabolism has been studied extensively in rodents. The current obesity studies are an extension of the rodent work with a population of people that can be studied longitudinally as well as cross-sectionally. Initially we have focused on the adult population characterizing the adipose cell size and number of this population, and have shown that after cell size increased beyond a certain point, any further increase in adiposity is accounted for by an increase in adipose cell number. In addition, comparisons between Pimas and Caucasians regarding the generating of obesity are being made to establish whether any differences in adipose cell metabolism between and two populations can be elucidated. We have also studied the effect of fasting as a function of obesity to determine whether the ability to mobilize fat can be correlated with obesity and adipose cell size. Finally, we are planing a lonitudinal study of the children and adolescents of the Pima population to determine whether the changes in adipose cell size andnumber correlate with the appearance of insulin resistance in this population. A better understanding of this relationship if important for both the study of obesity and diabetes.

ANNUAL REPORT FOR THE

EPIDEMIOLOGY AND FIELD STUDIES BRANCH

The EFSB has two Sections (Southwestern Field Studies and Biometry). The Phoenix Clinical Research Section (PCRS) also works in close collaboration with the Branch, although organizationally it is a component of the Digestive Diseases Branch. The major work of each of these sections is devoted to diseases particularly prevalent in the American Indian populations, especially diabetes mellitus. In addition to the studies of the etiology, natural history and pathogenesis of diabetes in the American Indian, the Branch also conducts and collaborates with other institutions such as the World Health Organization and South Pacific Commission on diabetes activities elsewhere and it is hoped that the activities may continue. These activities are summarized in the individual project reports.

During the past year Dr. W. C. Knowler was named Deputy Chief of the EFSB while continuing to serve as Chief of the SFSS. Other significant developments include the physical transfer of the Branch and its sections from leased office space to renovated space on the campus of the Phoenix Indian Medical Center, where the PCRS is located.

The effect of diminishing appropriations has been noteable during this year. Activities relating to the epidemiology of arthritis have been substantially reduced because the position of the medical officer in charge of many of these investigations was lost in the cut-backs and there was a major reduction in collaborative diabetes and obesity related studies because a major contract with the University of Texas Health Sciences Center in Dallas was terminated. Thus, the scope and extent of the investigations relating to diabetes and obesity were considerably reduced. In addition, a contract with Case Western Reserve University for data analysis was terminated, placing additional burdens on the Biometry Section. These cut-backs have not yet appreciably influenced the reported activities and publications, but their effect on the range of activities will be more obvious in the next fiscal year.

Biometry Section

The Biometry Section serves as a resource to the Epidemiology and Field Studies Branch, the Southwestern Field Studies Section (SFSS) and the Phoenix Clinical Research Section. It provides computer services to the Branch and to the Phoenix Clinical Research Section as well as statistical consultation to these components. The scientific accomplishments are reported in the corresponding Section reports.

During the past year the Biometry Section programmers have made a number of improvements in the production programs involving the SFSS masterfile. Perhaps the most significant accomplishment was the creation of a random access, disk version of the entire SFSS masterfile. Provided with key files for some of the most common access filtering, it will permit the fastest masterfile access that has ever been available. The masterfile programs have been patched around two major disabilities that threatened to render the current system dysfunctional: The original file structure permitted a maximum of nine biennial examinations and a maximum of 9999 subjects on the entire file. Both problems have been solved well enough to permit indefinite continued use of the present programs. Several of the routine listings derived periodically from the masterfile--the Drivers' list as an example - have been significantly improved in content, readability, and usefulness. A measurable amount of forms revision has already been accomplished. Further progress is somewhat dependent on SFSS's updating the protocol for the Sacaton Clinic. The Pregnancy Data Base, now in its second year, is at a fairly high level of dependability. Additional changes will incorporate access to additional files and will bring the programs up to higher standards of Structured Programming.

Increasing time in the Section is being devoted to statistical consulting and research. Log-linear models need to be explored for their ability to model epidemiological problems in the SFSS. Research is needed to determine the appropriateness of Repeated Measures models for analysis of certain patterns of time-course data characteristically required for analysis of PCRS data.

The Biometry programmers have begun to acquire familiarity with contemporary Structured Programming techniques. Much additional training and experience is required to achieve competence in Structured disciplines. The programmers have had to spend increasing amounts of time keeping up with, and familiarizing themselves with, the relentless and seemingly more rapid changes in the computer system and in user software in Bethesda. The programmers' familiarity with the system is taken for granted, but the time required for maintaining that competence has increased sharply in the past two years.

The Section has analyzed the basic requirements of a state-of-the-art Data Base management system appropriate to the needs of the file creation, updating and access. Identification and implementation of such a system (almost certainly an existing one) will be pursued as time and other resources permit.

Southwestern Field Studies Section

The Southwestern Field Studies Section has continued to investigate the determinants of diabetes and its complications primarily from the ongoing prospective study of the natural history of diabetes in the Pima Indian population.

Further progress has been made in elucidating the causes of diabetes. The associations of diabetes with HLA type and with polymorphism in the region of the insulin gene suggest that diabetes susceptibility genes are on at least two chromosomes. This evidence for a polygenic mode of inheritance, evidence that the intrauterine environment plays a role in the subsequent development of obesity and diabetes in the offspring, and our previous findings of a strong relationship of obesity and diabetes development emphasize the complex set of factors which predispose to diabetes mellitus. The discoveries of probable genetic markers and nongenetic risk factors for diabetes in the Pimas are important steps toward understanding the causes of noninsulin dependent diabetes.

The findings from the longitudinal diabetes study, together with collaborative projects with the Phoenix Clinical Research Section, emphasize the importance of insulin resistance and hyperinsulinemia in the genesis of diabetes in this population. The genetic and nongenetic components of these abnormalities are important areas of continuing research.

Complications of diabetes remain important areas of study. In addition to the well-known micro- and macro-vascular complications under study for many years, attention has been focused on cataract, which has been found to be an extremely common disease in the Pimas, who may have a rate of cataract surgery higher than any yet reported. What extent this high rate is due to diabetes or to other undetermined causes is an important area for further research.

Studies continue on the natural history of rheumatoid arthitis, osteoarthritis, and ankylosing spondylitis. Biennial examinations include physical examinations of the joints, radiography, serology, and collection of other clinical data.

In view of the high prevalence of diabetes, diabetes complications, arthritis, cholelithiasis and the high death rates in the population, the Pima Indians continue to suffer from a heavy burden of ill health and remain a worthy population for continued medical research. The studies with the Pimas continue to provide an important resource, not only for direct research findings, but also because knowledge and experience gained with the cooperation of the Pimas is applied to other research, such as diabetes epidemiology programs throughout the world and the Health and Nutrition Examination Survey of cholelithiasis in Hispanic Americans for which the Chief of the Section serves as an advisor.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 69000-17 EFS

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Diabetes Mellitus and Other Chronic Diseases in the Gila River Indian Community

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W.C. Knowler	Chief	SFSS,NIADDK
OTHER:	P.H. Bennett	Chief	EFSB,NIADDK
	D.J. Pettitt	Assistant Chief	SFSS,NIADDK
	M.J. Carraher	Staff Physician	SFSS,NIADDK
	E.J. Ballintine	Clinical Director	IRP,NEI
	D.L. Mann	Senior Investigator	IB,NCI
	B. Vasquez	Staff Fellow	PCRS,NIADDK
	W.J. Butler	Computer Systems Analyst	BS,NIADDK
	H.R. Baird	Acting Chief	BS,NIADDK
	G.L.Addie	Administrator	EFSB,NIADDK

COOPERATING UNITS (if any) IHS Hospitals, Sacaton & Phoenix, AZ; K. Aleck, UNM; N.B. Rushforth, Case Western Univ.; R.C. Williams, ASU; R.Rate, IHS, Keams Canyon, AZ; I.R. Schwab, UC San Francisco; M.A. Permutt, Washington Univ.

LAB/BRANCH
Epidemiology and Field Studies Branch

SECTION
Southwestern Field Studies Section

INSTITUTE AND LOCATION
NIADDK, NIH, Phoenix, Arizona 85014

TOTAL MANYEARS: 11.1	PROFESSIONAL: 3.7	OTHER: 7.4

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The genetic and environmental determinants of noninsulin dependent diabetes mellitus and the natural history of its vascular complications are being studied in an Amerindian community. The study also includes other diseases common in the study population, such as cataract, cholelithiasis, several types of arthritis (see Project Z01 AM 69009-17), and adverse outcomes of pregnancy (Project Z01 AM 69001-13). The project provided evidence for genetic determinants of diabetes on two chromosomes and for both intrauterine and postnatal environmental risk factors for diabetes. Insulin resistance and/or hyperinsulinemia appear to be important initial abnormalities in the development of diabetes. Eye examinations and retinal photography are used in the study of risk factors for diabetic retinopathy and other eye diseases such as cataract.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 69001-13 EFS

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Complications and Outcome of Diabetic and Prediabetic Pregnancies

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D.J. Pettitt	Assistant Chief	SFSS, NIADDK
OTHER:	H.R. Baird	Acting Chief	BS, NIADDK
	P.H. Bennett	Chief	EFSB, NIADDK
	W.C. Knowler	Chief	SFSS, NIADDK
	M.J. Carraher	Staff Physician	SFSS, NIADDK

COOPERATING UNITS (if any)
Indian Health Service
Dr. Kirk Aleck, Department of Pediatrics, University of New Mexico
Dr. M.R. Forman, NICHD

LAB/BRANCH
Epidemiology and Field Studies Branch

SECTION
Southwestern Field Studies Section

INSTITUTE AND LOCATION
NIADDK, NIH, Phoenix, Arizona 85014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.00	1.15	0.85

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of the project is to determine the effects of diabetes mellitus and prediabetes on the complications and outcome of pregnancy in women in the Gila River Indian Community and on the subsequent growth and development of their children.

The diabetes status of every woman is determined at each pregnancy as well as at two-yearly follow-up examinations. The characteristics of women who have diabetes during the pregnancy (diabetics) are compared to those of women who are normal during the pregnancy and subsequently develop diabetes (prediabetics) or remain normal (nondiabetic). The offspring from these pregnancies are followed at two-yearly intervals after the age of 5 years. Offspring of diabetics were found to have a higher prevalence of obesity and of diabetes than did offspring of nondiabetics or of prediabetics. These differences persist for at least 25 years. This suggests that an abnormal intrauterine environment has lasting effects on the offspring.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 69006-12 EFS

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Gila River Indian Community Autopsy Study

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P.H. Bennett	Chief	EFSB, NIADDK
OTHER:	W.C. Knowler	Chief	SFSS, NIADDK
	D.J. Pettitt	Assistant Chief	SFSS, NIADDK
	M.J. Carraher	Staff Physician	SFSS, NIADDK

COOPERATING UNITS (if any) D. Kuban, Pathology Dept., Phoenix Indian Medical Center; P. LeCompte, Clinical Professor Emeritus, Harvard Medical School; L. Klein, Professor of Orthopedic Surgery and Biochemistry, Case Western Reserve Univ.; L. Orci, Chairman, Dept. of Morphology, University of Geneva, Switzerland

LAB/BRANCH
Epidemiology and Field Studies Branch

SECTION
Southwestern Field Studies Section

INSTITUTE AND LOCATION
NIADDK, NIH, Phoenix, Arizona 85014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.45	0.25	0.20

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The postmortem characteristics of Pima Indians of the Gila River Indian Community are investigated so that findings in subjects with and without diabetes mellitus can be correlated with studies in living subjects.

Postmortem examinations are performed on members of the Gila River Indian Community whenever possible, regardless of whether the subjects have prior evidence of diabetes. Tissues are examined by light and electron microscopy and the histology related to the clinical findings in the same subjects. The histology of the pancreatic islets of Langerhans is being studied in detail to describe the changes that take place in diabetes. The distribution of cell types varies considerably between different regions of the pancreas suggesting regional variation in function.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 69009-17 EFS

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Natural History of Arthritis and Rheumatism on the Gila River Indian Community

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P.H. Bennett	Chief	EFSB, NIADDK
OTHER:	D.J. Pettitt	Assistant Chief	SFSS, NIADDK
	W.C. Knowler	Chief	SFSS, NIADDK
	M. Carraher	Medical Officer	SFSS, NIADDK

COOPERATING UNITS (if any)
R.C. Williams, Arizona State University
H. Morse, R.G. Rate, M.D. Bonnell, Keams Canyon Service Unit, IHS
T. Kuberski, Formerly EFSB

LAB/BRANCH
Epidemiology and Field Studies Branch

SECTION
Southwestern Field Studies Section

INSTITUTE AND LOCATION
NIADDK, NIH, Phoenix, Arizona 85014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.20	0.85	3.35

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The development and progression of various forms of arthritis is being investigated in American Indian populations. The patterns of joint disease will be examined to determine if distinctive patterns of degenerative joint disease can be recognized, and the prognostic significance of various factors related to rheumatoid arthritis and ankylosing spondylitis determined.

The study which includes clinical radiographic and serologic studies of joint disease is a part of a prospective study of the Gila River Indian Community where studies of diabetes mellitus and other diseases are also being undertaken.

The frequency of Reiters Disease and ankylosing spondylitis and their relationship to HLA, and the possible role of infection in producing these disorders in Navaho and Hopi Indians have been investigated.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 69013-01 EFS

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Diabetes, Arthritis, and other Metabolic Diseases in the Pacific Region

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P.H. Bennett	Chief	EFSB, NIADDK
OTHER:	T. Kuberski	Medical Officer	EFSB, NIADDK
	B.V. Howard	Associate Chief	PCRS, NIADDK

COOPERATING UNITS (if any) WHO collaborating Centre for the Epidemiology of Diabetes Mellitus, WHO Collaborating Centre for Virus Reference and Research for the Pacific Region, Melbourne, Australia; Pacific Biomedical Research Center, Univ. of Hawaii; Depts. of Health, Cook Islands, Nuie, Vanuatu and New Caledonia, South Pacific Commission.

LAB/BRANCH
Epidemiology and Field Studies Branch

SECTION
Southwestern Field Studies Section

INSTITUTE AND LOCATION
NIADDK, NIH, Phoenix, Arizona 85014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The prevalence of diabetes and its associated complications have been assessed in several Pacific populations, including Polynesians and Melanesians, living traditional ways of life and in highly urbanized and westernized circumstances. Substantially lower frequencies of diabetes were found in the traditional circumstances and attempts to determine factors responsible for these differences, such as genetic background, physical activity and obesity, have been made.

The fortuitous occurrence of epidemic polyarthritis, caused by Ross River virus infection, led to a detailed description of the disease, including redefinition of the incubation period and the discovery of new vectors for the disease. A follow-up study was conducted to determine if infection during pregnancy resulted in fetal or congenital complications.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM70500-04 CMO

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Studies of Insulin's Regulation of Glucose Transport and Metabolism

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Samuel W. Cushman	Associate Chief	CMOS, NIADDK
OTHER:	Ian A. Simpson	Visiting Associate	CMOS, NIADDK
	Lawrence J. Wardzala	Staff Fellow	CMOS, NIADDK
	Chih-Chen Wang	Visiting Fellow	CMOS, NIADDK
	Ulf Smith	Visiting Scientist	CMOS, NIADDK
	Lester B. Salans	Chief	CMOS, NIADDK

COOPERATING UNITS (if any) Diabetes Branch, NIADDK, NIH; Institute of Physiology, University of Aarhus, Aarhus, Denmark; Metabolic Unit, Endocrine Institute, Rambam Medical Center, Haifa, Israel; Phoenix Clinical Research Section, NIADDK, NIH, Phoenix, AZ; Department of Biochemistry, School of Medicine, University of

SECTION
Cellular Metabolism and Obesity

INSTITUTE AND LOCATION
NIADDK, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
7.65	4.65	3.00

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The mechanism of insulin's interaction with its specific receptors in the plasma in the plasma membrane of the rat adipose cell is under investigation through an examination of insulin and insulin receptor internalization. A further characterization of the insulin-induced translocation of glucose transport systems from a large intracellular pool to the plasma membrane in the rat adipose cell is in progress through an examination of the membrane species comprising the intracellular pool, and of the chemical and immunological nature of the rat adipose cell glucose transporter. The role of this translocation process in insulin's stimulatory action on glucose transport in rat diaphragm and human adipose cell has now been suggested. A possible mechanism of insulin resistant glucose transport in the adipose cell in the aging rat model of obesity, with high fat:low carbohydrate feeding in the rat, and in the streptozotocin-induced diabetic rat model of insulin-dependent diabetes mellitus has been established with the demonstration of a relative depletion of the intracellular pool from which glucose transport systems are translocated to the plasma membrane in response to insulin in all three of these pertubed metabolic states.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM70501-04 CMO

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Insulin-Cell Interactions

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Ian A. Simpson	Visiting Associate	CMOS, NIADDK
OTHER:	Chih-Chen Wang	Visiting Fellow	CMOS, NIADDK
	Samuel W. Cushman	Associate Chief	CMOS, NIADDK
	Lester B. Salans	Chief	CMOS, NIADDK

COOPERATING UNITS (if any) Jose A. Hedo, Diabetes Branch, NIADDK, NIH; Ole Sonne, Institute of Physiology, University of Aarhus, Aarhus, Denmark

LAB/BRANCH

SECTION
Cellular Metabolism and Obesity

INSTITUTE AND LOCATION
NIADDK, Bethesda, MD 20205

TOTAL MANYEARS: 2.20	PROFESSIONAL: 1.70	OTHER: 0.50

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Insulin's interatction with its specific receptors in the plasma membrane of the adipose cell, is complex. The role of ^{125}I-insulin and insulin receptor internalization in the mechanism of this interaction is under investigation. At 37°C, insulin binding to intact isolated rat adipose cells is associated with the rapid appearance of intact insulin in both endoplasmic reticulum- and Golgi-enriched membrane fractions. Concomitantly, insulin receptors are lost from the plasma membrane fraction and appear in the Golgi fraction. Steady state subcellular distributions of insulin and its receptor are achieved within 30 min. These redistribution processes are insulin concentration dependent, and are inhibited at 16°C. The redistribution of the insulin receptor can be observed by measruing insulin binding to the receptor directly, by photoaffinity labeling the 138K receptor subunit, or by immunoprecipitating both the 138K and 95K receptor subunits following their iodination using lactoperoxidase. The receptor in the Golgi fraction, however, is accessible to insulin only in the presence of detergent. Thus, insulin induces a rapid, and insulin concentration- and temperature-dependent internatlization of its own receptor from the plasma membrane to a Golgi-enriched membrane fraction through an endocytic-like process.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM70502 -04 CMO

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Insulin's Regulation of Glucose Transport

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Samuel W. Cushman	Associate Chief	CMOS, NIADDK
OTHERS:	Ian A. Simpson	Visiting Associate	CMOS, NIADDK
	Lawrence J. Wardzala	Staff Fellow	CMOS, NIADDK
	Ulf Smith	Visiting Scientist	CMOS, NIADDK
	Lester B. Salans	Chief	CMOS, NIADDK

COOPERATING UNITS (if any) Eddy Karnieli, Metabolic Unit, Endocrine Institute, Rambam Medical Center, Haifa, Israel; James E. Foley, Phoenix Clinical Research Section, NIADDK, NIH, Phoenix, AZ; Thomas J. Wheeler, Depavment of Biochemistry, School of Medicine, University of Louisville, Louisville, KY

LAB/BRANCH

SECTION
Cellular Metabolism and Obesity

INSTITUTE AND LOCATION
NIADDK, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.05	2.55	2.50

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A specific D-glucose-inhibitable cytochalasin B binding assay for quantitating the number of glucose transport systems in subcellular membrane fractions has previously been used to demonstrate that insulin stimulates glucose transport in the isolated rat adipose cell primarily through the translocation of glucose transport systems from a large intracellular pool to the plasma membrane. This intracellular pool is associated with the cell's low-density microsomal membranes. This assay has now been used to demonstrate that the intracellular pool may comprise a specialized membrane species and that intracellular glucose transport systems may be chemically processed during their cycling to the plasma membrane in response to insulin. In addition, the rat adipose cell's intracellular glucose transport systems appear to be immunologically similar to the human erythrocyte glucose transporter with a MW_r of roughly 45,000 daltons, and fully functional when reconstituted into liposomes. Furthermore, insulin may act at a step in the translocation process whereby plasma membrane-associated glucose transport systems become functional. Insulin also appears to stimulate glucose transport in rat diaphragm and the isolated human adipose cell through this same translocation mechanism.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM70503-04 CMO

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Alterations in Insulin's Action in Obesity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Samuel W. Cushman	Associate Chief	CMOS, NIADDK
OTHER:	Lester B. Salans	Chief	CMOS, NIADDK

COOPERATING UNITS (if any) Paul J. Hissin, Department of Chemistry, Mount Sinai Hospital, New York, NY.

LAB/BRANCH

SECTION
Cellular Metabolism and Obesity

INSTITUTE AND LOCATION
NIADDK, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.15	0.15	0.00

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Increasing adipse cell size in the aging rat model in obesity is accompanied by a marked reduction in insulin's ability to stimulate glucose metabolism. Since a fundamental action of insulin in regulating glucose metabolism is its stimulatory effect on glucose transport and since insulin appears to enhance glucose transport by triggering a rapid and reversible translocation of glucose transport systems from a large intracellular pool to the plasma membrane, the effects of increasing cell size on this pool and the translocation process in the isolated adipose cell have been examined in relation to glucose transport activity in the intact cell. With a 10-fold increase in cell size, insulin's ability to stimulate L-arabinose transport in intact cells and to increase the concentration of glucose transport systems in the plasma membrane fraction prepared from these cells practically disappears. At the same time, the basal concentration of glucose transport systems in the low-density microsomal membrane fraction prepared from the enlarged cells is decreased by 80%. Thus, adipose cellular enlargement is accompanied by a markedly reduced glucose transport response to insulin which appears to be the consequence of a relative depletion of glucose transport systems in the intracellular pool.

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PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Alterations of Adipose Cell Function to the Genetically Obese Zucker Fatty Rat

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Samuel W. Cushman	Associate Chief	CMOS, NIADDK
OTHER:	Lester B. Salans	Chief	CMOS, NIADDK

COOPERATING UNITS (if any)

LAB/BRANCH

SECTION
Cellular Metabolism and Obesity

INSTITUTE AND LOCATION
NIADDK, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.00	0.00	0.00

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Terminated.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM70505-04 CMO

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Role of Dietary Carbohydrate and Rat in Modifying Insulin's Action

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Samuel W. Cushman	Associate Chief	CMOS, NIADDK
OTHER:	Lester B. Salans	Chief	CMOS, NIADDK

COOPERATING UNITS (if any) Paul J. Hissin, Department of Chemistry, Mount Sinai Hospital, New York, NY.

LAB/BRANCH

SECTION

Cellular Metabolism and Obesity

INSTITUTE AND LOCATION

NIADDK, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.15	0.15	0.00

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

High fat:low carbohydrate relative to high carbohydrate:low fat feeding is associated with a marked reduction in insulin's ability to stimulate glucose transport in the isolated rat adipose cell. Since insulin appears to enhance glucose transport by triggering a rapid and reversible translocation of glucose transport systems from a large intracellular pool to the plasma membrane, the effects of high fat:low carbohydrate feeding on this pool and the translocation process in the isolated rat adipose cell have been examined. With high fat:low carbohydrate feeding, insulin's ability to stimulate 3-0-methylglucose transport in intact cells and to increase the concentration of glucose transport systems in the plasma membrane fraction prepared from these cells is decreased by 55% and 41%, respectively. At the same time, the basal concentration of glucose transport systems in the low-density microsomal membrane fraction prepared from cells of high fat:low carbohydrate-fed rats is decreased by 47%. Thus, the reduced glucose transport response of the rat adipose cell to insulin with high fat:low carbohydrate feeding appears to be the consequence of a relative depletion of glucose transport systems in the intracellular pool.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM70506-02 CMO

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Alterations in Insulin's Action in Insulin-Dependent Diabetes Mellitus

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Samuel W. Cushman	Associate Chief	CMOS, NIADDK
OTHER:	Lester B. Salans	Chief	CMOS, NIADDK

COOPERATING UNITS (if any) Eddy Karnieli, Metabolic Unit, Endocrine Institute, Rambam Medical Center, Haifa, Israel.

LAB/BRANCH

SECTION

Cellular Metabolism and Obesity

INSTITUTE AND LOCATION

NIADDK, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.10	0.10	0.00

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The streptozotocin-induced diabetic rat model of insulin-dependent diabetes mellitus is associated with a marked reduction in insulin's ability to stimulate glucose transport in the isolated rat adipose cell. Since insulin appears to enhance glucose transport by triggering a rapid and reversible translocation of glucose transport systems from a large intracellular pool to the plasma membrane, the effects of streptozotocin-induced diabetes on this pool and the translocation process in the isolated adipose cell have been examined. With the induction of diabetes, insulin's ability to stimulate 3-0-methyglucose transport in intact cells and to increase the concentration of glucose transport systems in the plasma membrane fraction prepared from these cells is decreased by 55% and 40%, respectively. At the same time, the basal concentration of glucose transport systems in the low-density microsomal membrane fraction prepared from cells of the diabetic animals is decreased by 45%. Thus, the reduced glucose transport response of the adipose cell to insulin in the streptozotocin-induced diabetic rat appears to be the consequence of a relative depletion of glucose transport systems in the intracellular pool.

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